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THE CHEMISTRY OF HEREDITY

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This book is dedicated to the enlightened programs of research grants in our country whose support made possible most of the recent discoveries in the field of the chemistry of heredity

INTRODUCTION

THE very thought of reading a book about the *Chemistry of Heredity* is likely to encounter a great deal of resistance The word "Chemistry is often inimical and reminds one of a lot of difficult or boring moments in one s school days. And why study heredity at all? It s with us, like the weather and nothing can be done about it.

Well, perhaps something can be done about it. And chemistry if properly seasoned, need not be impalatable. And perhaps the whole subject affects the progress of medicine more than one thought

The first reason to study the chemistry of heredity is plain curiosity here is an important natural mechanism and so little is known about it. Especially after the second world war this thought became so irritating and humiliating to basic scientists that they decided to pursue the field and comparatively swift progress has been achieved alas, the field even became fashionable.

But the problem of the chemistry of heredity also has most important practical applications, for the practitioner as well as for the researcher Let s look into these applications.

I. HUMAN HEREDITY

A number of diseases or defects, of previously unknown origin, have been found to be hereditary This number increases rapidly as the family records of cases are being studied. In a few instances (to be discussed later) the bio-

chemical defect underlying the disease has been well traced others still remain a challenge to the researcher but if the disease is known to be hereditary there is a good chance of mapping the reactions involved and trapping the defect, usually as a block in an enzymatic system. Once the biochemistry of the defect is known, an intelligent treatment can be (and has been) proposed

A related field is that of the hereditary susceptibilities to disease. Of these, the problem of hereditary susceptibility to various types of cancer holds at present a great deal of interest. While the statistical studies in human populations did not result yet in a unanimous opinion on this subject, there is absolutely no doubt that in laboratory animals (mice) the susceptibility to some types of cancer can be hereditary The chemical basis of this hereditary defect is as yet completely unknown, the problem may have to wait for the progress in the understanding of cancer in general

II. CANCER

The problem of cancer appears to be closely connected with that of the chemistry of heredity In the fruit fly (*Drosophila*) and in certain fishes some forms of cancer are hereditary The genes for it have been located in the chromosomes, like any other genes, and there is no reason why they should not follow the general make up of the gene. This alone suggests that the study of the *chemistry of the gene* is not without interest

One of the respected hypotheses of the origin of some types of cancer (also in man) suggests that the cancerous cell occurs as a mutant of a somatic cell if so then the *chemistry of mutation* too is of considerable interest.

Another hypothesis indicates that a virus may be a causative agent in some types of cancer As it will be shown subsequently the infective agent of the virus (the "gene" of

the virus) is a chemical substance closely related to the substance of the genes of the host, the interaction of the two biochemical systems is again an important problem in the field of chemistry of heredity

Finally the mode of action of some carcinogenic agents, the elucidation of the upsetting of biochemical processes in the cancerous cell, the modern chemotherapy of cancer based on inhibition of synthesis of genetical material of cancerous cells, are all problems in the field of chemical genetics.

III. PATHOGENIC MICROORGANISMS

If the problem of infectious diseases caused by bacteria and other fungi appears to us less menacing than it did to the previous generation, it is only because temporarily thanks to antibiotics, we have gained the upper hand in the fight against these microorganisms. As we know the microorganisms fight back, by mutating to strains resistant to antibiotics so that new antibiotics have to be constantly discovered. What if we slow down, or worse still, reach the bottom of the barrel?

The study of mutations of microorganisms to antibiotic resistance is one of the subjects in the rapidly expanding field of research, *microbial genetics* The chemistry of the gene in general, and of mutation in particular the transmission of genetic material from one cell to another are again at the base of all the problems in this field.

The chemical substances carrying the heredity of the *viruses* have been extracted and are open to chemical study This study has suggested ways of how to interfere with the normal process of synthesis of these substances so as to inhibit the multiplication of the virus. The important problem of mutation of viruses to the strains which are more virulent (epidemiology) or which are attenuated (for vac

cines) is also essentially a problem in the chemistry of mutations. Recently the study of the chemistry of the viral "genes" has led to the laboratory production of viruses with known chemical changes in their genes; thus viruses which never existed in nature have been obtained, a development of as yet unpredictable consequences.

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The rapid progress in the field of the chemistry of the genes has been greatly helped by the fact that the chemical basis of the genes is essentially similar in all living entities, down to the simplest viruses. This obliging circumstance allows the biochemist to work with genes of convenient, fast multiplying organisms such as bacteria, and yet draw valid conclusions concerning the chemistry of human genes. One is tempted to speculate that this is because these chemical substances (or their prototypes) determining the heredity must have been the first heralds of life, indeed one cannot visualize a living entity which would not carry its own heredity. Thus, of all the perplexing systems, the one concerned with genetics is considered (by the geneticists) the most essential for the definition of life.

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THE CHEMISTRY OF HEREDITY

Chapter I

MECHANISMS OF HEREDITY

If we were asked to design, in broad terms, a complete mechanism for the orderly transmission of human heredity by using simple logic, how would we go about it?

Perhaps at first we would make sure that we have a way of registering the information for determining how the next human generation would be like. We would want to be sure that such information is never lost thus, whatever vehicle we choose for carrying this information, it would have to be a *stable* one. After all, it takes many thousands of years to accumulate the biological experience of the race and it is a very painful process, involving the elimination of the less fit we would not want to lose this experience in a moment just because the information carrier was unreliable. Thus, the first essential elements in our design would be some rather stable, transmissible information carriers, which at various times have been called genes, genetic determinants or heredity determinants. The name "determinant" implies that the Thing is not merely passive but actually determines what the next generation will look like

It is not enough for the heredity determinants to be passed to the next generation (through the fertilized egg) for there are a great many cell divisions between the egg

The gene may mean different things to different scientists; however since the term is so well entrenched in the literature, it will be used in this book side by side with the better defined term "heredity determinant.

and the mature individual and every time a cell divides, the heredity determinants would get diluted twofold, until practically none would be present in the cells of a mature individual So if we want the information to be passed undiluted and unchanged to one more generation, we should make sure that our heredity determinants know how to duplicate themselves (replicate) unchanged, preferably every time a cell divides this way they will always be present undiluted at a moment's notice

Thus, the heredity *determinants are the first system* which we need. But this, of course, is not sufficient We know that the genes determine everything in the organism the blue color of the eyes as well as the milk secretion. But we would not want the genes themselves to have pigment or to secrete milk. What we need, then, is an intermediary mechanism which carries the orders from the genes to the site of execution of these orders, that is the final chemical reactions. These reactions may take place anywhere in the cell, sometimes even on the outer surface of the cell membrane surely it would not be safe for the cells to keep there their most valuable records, the genes. Our design would be wiser if the genes were located in some vault buried deep within the cell, such as the nucleus. But that of course, will necessitate the intermediary mechanism we just mentioned To realize how much such a mechanism is needed, one may recall that in some cells, notably motor neurones, the tip of the axon, where some gene-controlled reactions take place, may be four feet away from the nucleus!

We do not know much what to require of such an *intermediary mechanism* we are now designing but one thing we do know we want this mechanism to be practically *unidirectional* We allow changes in the genes to be transmitted to the final chemical reactions otherwise there

would be no evolution. But we do not want the changes in the final chemical reactions to be transmitted back to the genes, because these chemical reactions may be influenced by the environment, and its changes. To give an example: a mechanical injury to the gonads may upset many of cellular reactions, still we want the genes to remain unchanged so that our children be born without injury to their gonads.

This, then, was the second system which we need: the intermediary mechanism. Finally the third system required would be the final gene-controlled chemical reactions of the cell, which we may call the *peripheral reactions*. This should be a very intricate system, including practically all that is going on in the cell, because probably nothing can go on without an active or silent approval of the genes.

If we now examine the mechanisms of heredity actually existing in nature, we will find that they correspond rather well to what we have designed in general terms using simple logic. Our present knowledge of each of these systems leaves very much to be desired, in fact, we are just entering the vestibule of infinity. Nevertheless, these three systems are very real, as illustrated briefly by the following examples.

There is a substance, called transforming principle, which somehow can carry the genetic information from one bacterial cell to another. More will be said about this substance in the next chapter: here it suffices to mention that the chemical nature of the substance is well known and that the substance can perform its rôle (carrying the genetic information) all by itself without any necessity of accessory substances. To be sure, we do not know that the substance can determine heredity until the substance has found its way into the living cell but this is not paramount. the main point is that the substance can be extract

Which is science' greatest charm.

ed uninjured, purified and thus separated from all the other systems, and that this separation does not change the quality or quantity of the genetic information. This is, then, an example of an independent mechanism which we have called the first system, the heredity determinants.

An interesting example of the second, or intermediary system, which carries the orders of the genes through the cell, is furnished by an alga, *Acetabularia*. The organism looks like a slender mushroom about one inch high, with a rootlet and a shank; on further cultivation, a small green cap is formed on top of the shank. This alga is interesting because it consists of a single cell and has a single nucleus normally situated in the rootlet. Suppose now that we cut off the nucleus-carrying rootlet of a young alga which has not yet developed the cap. One could think that since the nucleus with the genes in it has been removed, the plant will not know how to make the cap since the shape and the color of the cap must be gene-determined. Yet the alga *does know* and makes itself a perfect green cap; in fact, for at least two weeks after cutting the synthesis of protein proceeds at the undiminished rate. Where was the genetic information? It is believed that it has been passed from the nuclear genes to the information-carrying second system in the cytoplasm; this system can now perform, independently from the first system (heredity determinants) which has been literally cut off.

The separate third system was actually the earliest one to draw attention of biochemists; in fact for many years the expression "chemical genetics" meant only a branch of genetics concerned with the effects of mutations on the peripheral reactions. This system is also the easiest to separate from the others. The gene-controlled final reactions in the cell are usually enzymatic reactions; the enzymes can be extracted from the cell, purified and often even crystal-

lized they can be made to work *in vitro* on their appropriate substances, which are either extracted from the cell or synthesized in the laboratory the conditions existing in the cell (pH temperature) can also be imitated Thus the entire peripheral enzymatic reaction can often be re-created in the test tube, even though the heredity determinants and the information carrying systems are no longer there.

It may be useful to correlate the biochemical concept of the three systems with the terms genotype" and phenotype used in genetics. The genotype is the genetic make up clearly the chemical basis of it is the first system (heredity determinants) The phenotype is the physical makeup the way the individual looks. But the way the individual looks depends on what *we* like to look at. The color of the eye is obviously a phenotype. But the chemist prefers to look at the chemical structure of the pigment this is phenotype, too So is the appearance of the chromosomes, and even the chemical structure of the gene for although an individual gene makes the law" for the cell, its chemical structure must also obey the laws dictated by all the other genes. Thus, the phenotype of an individual is the end product of the collaboration of all three systems with the environment.

Chapter II

HEREDITY DETERMINANTS AS CHEMICAL SUBSTANCES

In her writings nature does not use capital letters; it is up to us to distinguish the essential from the trivial.

In the previous chapter a mention was made, without any proof, that a chemical substance can serve as the heredity determinant. Such proposition is not obvious at all, and therefore in this chapter an evidence will be presented to support the idea.

The thought that one's genes were so superior because of a mere substance, was likely to be unpopular for a long time. Students of the history of science cannot fail to notice how often the issues most essential for our species were continuously avoided. Thus, in general the science of heredity which affects us more than astronomy was practically nonexistent until the second half of the nineteenth century although Mendel's conclusions (1866) were actually much easier to arrive at and to accept than those of Copernicus (1530) or of Harvey (1628). As late as 1872 Spencer writes: "We are obliged to confess that Life in its essence cannot be conceived in physico-chemical terms."

By the end of the first half of this century the foundations for chemical explanation of several biological phenomena had already been laid. However the phenomenon of heredity was not attractive to a chemist, perhaps because of a fear of the multitude of substances involved and their in

surmountable complexity indeed in contrast to simpler biochemical functions, nothing less than the whole organism, the whole cell or at least the whole chromosomal apparatus seemed indispensable as heredity determinant. The remains of this nebulous attitude still hamper research on the chemistry of heredity in a certain part of Eastern Europe But even after the acceptance of the hypothetical concept of a unit of heredity the gene, it was still not clear whether such a concept had a physical reality and even if the gene was real presumably it could be something other than a substance. True, the chemical substances are at the base of all living matter but the substances as such are not the only things which can carry information. Consider for a moment a charcoal sketch. the information that the artist is trying to convey to us is not in the nature of charcoal particles. The particles are all alike but what is specific and information-carrying is their *specific distribution* on the paper Now one knew for a long time that the chromosomes are somehow involved in the orderly transmission of heredity it was therefore natural to think that it is the organization of the chromosome, the specific distribution of unspecific, uninteresting molecules that is responsible for determining the heredity

Such an hypothesis was indeed proposed It was stimulated by the discovery of a phenomenon called the position effect (for a review see) the action of some genes proved to depend, to some extent, on their position among the neighboring genes. For example, in the fruit fly *Drosophila*, a chromosomal region may become inverted, or it may become transplanted (translocated) into another place in the chromosomes, such alterations may result in a change in gene action. However it has been found that this effect is by no means universal, even in *Drosophila*[1] This alone would speak against the concept of the position or distri

bution as being the universal basis of heredity determination. Actually the position effect can be explained as well by the hypothesis that the information is carried by chemical molecules provided that there is some kind of interaction between them a disturbance in the interaction may result in a change in action. Indeed, some sort of interaction or linkage between the information-carrying molecules in a chromosome has been recently reported But the most decisive blow against the hypothesis that the entire information is due to distribution of uninteresting molecules, was delivered by the discovery of the transforming phenomenon. In this phenomenon, the individual molecules were extracted from the chromosome, their specific distribution was completely destroyed since they were independently moving in the solution, and yet they still carried unchanged genetic information therefore, the molecules themselves must have been interesting But let us first describe the transforming phenomenon from the very beginning it is worthwhile to spend some time on this development since it represents a major break through in biological thoughts of our century

In 1928 Griffith reported that when mice were injected with living non-encapsulated (R) pneumococci mixed with heat killed encapsulated (S) ones, living S pneumococci were recovered from the animals. Of particular interest was the fact that the specific type of the S cells was the same as the type of the heat-killed cells rather than the type from which the living R cells were derived (by some previous mutation to the loss of capsule) Once the new feature (production of the capsule of a specific type) was established, it was retained and reproduced in subsequent generations as if a new gene had been added to the genetic makeup of the receptor cells, indeed, such transformed cells, when heat killed, could now serve to induce the

transformation of R cells exactly in the same way as did the original S cells.

Although this experiment was confirmed by other workers, it did not attract due attention. despite careful controls it still appeared possible that the seemingly heat-killed preparation contained some heat resistant bacterial forms so that the phenomenon was a revival of the S cells rather than the "transformation of the R cells. The possibility of a heat resistant virus also was not excluded.

In 1931 Dawson and Sia[13] reported that transformation can also be demonstrated *in vitro* These workers realized that the phenomenon affects only one out of many R cells and that it is necessary to depress the growth of this strain to give the few newly formed S cells a chance to multiply to the point of recognition. They therefore performed the experiment in the presence of an anti-R serum, today this method is still the preferred one when transforming the R cells into S cells.

Alloway was first to demonstrate that the presence of the whole donor" (S) cells is not necessary for the phenomenon. this investigator prepared cell free aqueous extracts of the heat-killed S cells, passed the extract through a bacterial filter and demonstrated that the filtrate is still capable of transforming the R cells into S cells. While this experiment excluded the presence of a non-filterable bacterial form, it did not exclude the filterable virus as the responsible agent, the experiment was merely suggestive that the agent is water-soluble

In 1944, Avery MacLeod and McCarthy[1] purified the extract further and found that the responsible agent (the transforming principle") has all the properties of a highly polymerized deoxypentose nucleic acid (DNA) this single finding laid the foundation for the study of the transforming principle.

The post war period witnessed several important contributions to our knowledge of the phenomenon and its ramifications. Besides pneumococcus, the transformation has been accomplished in *Hemophilus influenzae* (Alexander and Leidy 1950) these two bacterial species have since served as dependable tools for studying the transforming phenomenon *in vivo* and the behavior of the transforming principle *in vitro* The phenomenon has been also reported in many other bacteria (for two recent reviews see [7]) It was also found that the feature transferred (induced) in the transforming phenomenon is not limited to the production of capsular substances. Other transferable features include induction of fermentation of salicin, change in metabolism of glucose and lactic acid, production of mannitol phosphate dehydrogenase, production of several specific enzymes in the biosynthetic pathway of tryptophane or uronic acids [10] Still other features include the change in the *quantity* of polysaccharide produced in this case it was suggested that the genes involved form a series analogous to what is known in higher organisms as an *allelic* series. Still other transferable features, first reported by Hotchkiss[11] include the change from sensitivity to resistance to penicillin, sulfanilamide, streptomycin, erythromycin etc., and even a reverse change, from resistance to sensitivity This class of transformation phenomena is of particular interest because it involves gradations (one-step or multistep acquisition of resistance) which resemble closely the gradations acquired by a natural process, that is by spontaneous mutation.

Some scientists feel that, if given enough effort, probably every feature could be shown to be transferable, and that *ultimately the entire bacterial heredity will be accounted for by the extractable substance, namely* DNA Other scientists are more cautious perhaps there are some

heredity features carried by another substance? The evi dence that some features indeed are determined by some thing in the cytoplasm (though still under ultimate control of the nuclear genes) will be given in Chapter V when we will discuss so called cytoplasmic inheritance. However this auxiliary inheritance seems to be neither essential nor independent, and nowadays scientists in general agree that DNA is the only substance which enjoys the evidence for carrying the main" heredity of the bacterial cell.

As intimated above, when one looks at the results of transformation (acquisition of a capsule or acquisition of a resistance to antibiotics) one cannot help noticing their great similarity to ordinary mutations. Yet the mechanism of the two processes seems to be different in transforma tion we *add* a new molecule of heredity determinant, in mutation we *change* the existing one. Not all the scientists agree with this view some feel that since we do not know exactly what happens in the cell, we cannot exclude the possibility that the entering molecule of DNA does not add itself but merely changes the existing molecules, es pecially at the time of their replication

Whatever the details of the phenomenon, the possibility of working with the hitherto inviolable material of the cellular nucleus is of great importance the violation of the atomic nucleus by the physicists was louder but in a long run the tinkering with the genes may be of more conse quence. As a modest beginning one can already extract from the "donor" bacterial cells the heredity-carrying DNA, purify it like any other chemical substance, study its physical and chemical properties, subject it in the test tube to physical and chemical agents, and then introduce it into the receptor cell where it will still work and deter mine the heredity of this cell, like any other gene (for two recent reviews, see)

While this was going on in the field of bacteriology the scientists began to look for similar evidence in other living entities. Viruses, being simpler than bacteria, seemed admirable material and attracted much attention from basic scientists.

In 1951 Lederberg and Zinder noticed that when Salmonella cells with certain hereditary traits are lysed especially by weakly lytic bacteriophages (bacterial viruses) the lysate contains a filterable agent capable of inducing these traits in other Salmonella cells the phenomenon has been given the name transduction"[11]

The cause of the phenomenon was eventually found to be associated with a bacteriophage presumably serving as a carrier for the material which changes the heredity of the host (receptor) cells. The nature of this phenomenon is still not clear If the hereditary material transferred is a chemical substance (DNA) then the phenomenon can be called a special case of transformation (or vice versa) however if the transferred material is a bacterial chromosome or its part then the phenomenon may be of a different nature. Moreover for this study it should be stressed that the hereditary material, when outside of the cell, seems to be completely protected this indicates that the phenomenon does not lend itself to physico-chemical study of the material *in vitro* For this reason, the phenomenon, though undoubtedly very interesting will not be discussed further in this book

More insight into the chemical nature of the genes of viruses was furnished by the experiment of Hershey and Chase[1] These investigators grew bacteriophage in bacteria in the presence of radioactive sulfur and radioactive phosphorus. Sulfur is present in certain amino acids, therefore, the viral proteins having these amino acids became labelled with radioactivity On the other hand, phosphorus is pres-

ent in the nucleic acids and in this way the latter became labelled with radioactivity Next step was to infect the host cells with such doubly labelled phage and then to search for the presence of radioactive sulfur or phosphorus within the bacteria. if only the radioactive phosphorus were there, it would mean that only the nucleic acid of the phage penetrated into the host cell if only sulfur it would mean that only the proteins did. The result was that practically only the phosphorus was radioactive, clearly indicating that the infection meant the passage of essentially only nucleic acid of the phage into the host cell In addition, since the only nucleic acid found in phage is DNA, the conclusion is that the DNA alone is the infective material of the phage. This conclusion, now widely accepted, was another important development in our understanding of the chemistry of heredity

It must be noted that thus far the attempts to infect the host cell with DNA alone have been unsuccessful the proteins are still needed, as auxiliary substances for infection though they do not determine heredity However the work in this field is progressing a recent study[8] has shown that if one removes the cell wall from the bacterial host cell (forming a so-called protoplast") then the cell can become infected with a phage which is so injured as to become a half way form between pure DNA and the intact virus.

As will be discussed below the presence of DNA is in general a characteristic of larger viruses many smaller ones contain RNA as the only nucleic acid. Among those studied early were the plant viruses, first obtained in crystalline form by Wendell M Stanley[1] The achievement, for which Stanley received a Nobel Prize in 1946, had a most

The trace of protein, accompanying the DNA in its journey into the cell, is believed to hav merely an accessory rôle

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Two "hybrid" viruses resulted N_AP_B and N_BP_A, where N stands for nucleic acid, and P—for protein. The problem was now which of the two components will the hybrid obey upon reproduction? Will it follow the nucleic acid (N_AP_B to become N_AP_A and N_BP_A to become N_BP_B) or the protein (N_AP_B to become N_BP_B and N_BP_A to become N_AP_A)? The answer was unequivocal the hybrid always followed the *nucleic acid* component, the protein was immaterial for the determination of heredity Thus, the nucleic acids proved once more to be the sole carriers of genetic information

Were the nucleic acids alone sufficient for infection? This question was answered by the studies of Gierer and Schramm[15] and Fraenkel-Conrat[11] Gierer and Schramm's methods of separating the nucleic acid and the protein were such that the protein became destroyed in the process this appeared unfortunate since they could not recombine the two components as described above As it often happens in science, this unfortunate situation proved to be most fortunate when tested on a plant, the nucleic acid alone proved to be infective! Fraenkel-Conrat reexamined his two separated components and found that while the protein was indeed completely non infective, the nucleic acid had some infectivity

Stanley[3] goes as far as to say that the nucleic acid *is* the virus. To be sure, the complete virus is more infective than nucleic acid alone but the scientists feel that the rôle of proteins in improving the infectivity is merely accessory perhaps they serve as a protection against the enzymes attacking the nucleic acid, or perhaps they produce an improvement in virus attachment but as far as heredity is concerned, the nucleic acid is all that is necessary

Needless to say, scientists were now interested in finding a similar behavior for animal viruses The speed of devel

opments in this field was indeed remarkable, indicating that at last the weight of such discoveries was fully realized. Within two years, the infectivity of ribonucleic acid from Mengo and West Nile encephalitis virus (from Ehrlich ascites tumor cells) polio virus[3] and Eastern equine encephalomyelitis virus was reported. The finding of the infectivity of the polio virus RNA is of particular interest The infection with such RNA can now be accomplished in single layers of human cells in tissue culture The infected cells form dead spots (plaques) if the RNA is destroyed by the enzyme ribonuclease, there is no infection and the plaques are not formed (Figure 1) All these findings are too new for us to predict future development but undoubtedly they will put our knowledge of the corresponding diseases on a new logical level In particular the recognition that the infective agents are nucleic acids should help us to design safe vaccines in which the infective agent is destroyed as will be mentioned further in, our knowl

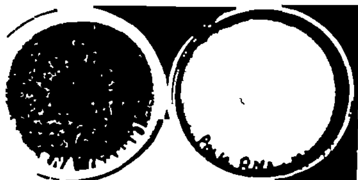


FIG. 1 Dead spots (plaques) produced by the infective ribonucleic acid (RNA) of the polio virus on the single layers of human cells in tissue culture. Left-active RNA right-RNA destroyed by enzyme ribonuclease (Courtesy Dr J Morgan Mountain)

edge of agents capable of destroying the nucleic acids is by now quite extensive.

In summary then, the heredity determinants of certain (usually smaller) viruses are ribonucleic acids, while those of larger viruses (bacteriophages) and of bacteria—deoxyribonucleic acids. What about higher organisms, especially man?

In all honesty it must be admitted right at the start that our evidence about the substance determining heredity in higher organisms is not as convincing as it is for viruses and bacteria

Transformation in higher organisms has not yet been proved though several laboratories are attempting to do it One *guesses* that the story must be similar because so many basic processes are similar in all living things; one has some *indirect* evidence that it is so and one has patience.

Let us look, however at this indirect evidence. The chromosomes, long recognized as the most essential part of the apparatus for the orderly transmission of heredity show the presence of a large amount of nucleoprotein, in this compound, the nucleic acid is of the deoxyribose type (DNA) (for a review see) The spermatozoon, which carries the entire heredity information the male is ever going to transmit to the next generation, consists of over 90 percent of nucleoprotein (on a dry basis) in it, the DNA content can be as much as 2/3 of the total.

The granules or bands of granules in the chromosomes which can be seen in many cells, most distinctly in the giant chromosomes of the salivary gland of the fruit fly *Drosophila* (Figure 2) consist again of nucleoprotein of the deoxyribose type. These granules or bands, demonstrable by the use of basic dyes or ultraviolet light, have been known to be associated with the actual gene loci. if the gene

According to unconfirmed reports it has been accomplished in ducks.

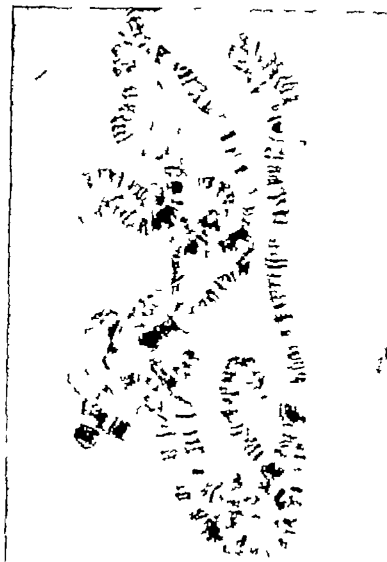


FIG. 2. Giant Chromosomes of the salivary gland of the fruit fly *Drosophila melanogaster* The cross-bands contain deoxyribonucleoprotein.

changes its position, so does the corresponding band. All these observations suggest that here, too, the nucleoprotein plays a very important rôle in the determination of heredity But after these observations the unanswered question remains: what carries the information the protein, the nucleic acid or the specific way the two are combined? Some insight into this problem can be gained by the analysis of these components: it has been found that the composition of DNA from spermatozoa is the same as that from the chromosomes of somatic cells of the same species (for a review see) In contrast to this, the composition of protein may change drastically in the chromosomes of spermatozoa the protein is a *protamine* whereas in the corresponding chromosomes of erythrocytes it is a *histone* a protein of a very different composition The replacement of histone by protamine appears to occur during an advanced stage of spermatogenesis[6] One instinctively feels that the substance which does not change (DNA) is a more reliable heredity carrier than the one (protein) which changes so drastically during the development for the same reason the bond between the DNA and the protein would not be a reliable register of the genetic information But an instinctive feeling is one thing and the scientific proof is something else again.

In 1949, Vendrely and Vendrely and subsequently Mirsky and Ris found that the *amount* of DNA per (diploid) cell is the same in all organs of the same species (Table I) in haploid cell (that is the cell with only one set of chromosomes) such as spermatozoon, this amount is fully half This constancy applies to DNA only· the RNA and protein content vary very much in spermatozoa and in thymus cells for example the amounts of the above substances are very small, in liver cells—very large. Now these findings already give some, though still indirect, evidence.

TABLE I

THE AMOUNT OF DEOXYRIBONUCLEIC ACID (DNA) IN CELLS OF DIFFERENT SPECIES

	DNA per nucleus, in 10^{-9} mg
FISHES	
Shark	6 67 5 46
Carp	3.2 3 49
Pike	1.7
Shad	0.94
AMPHIBIANS	
Amphiuma	168
Necturus	48.4
Frog	15 0
Toad	7.33
REPTILES	
Green Turtle	5.27
Water Snak	5.02
Alligator	4 98
BIRDS	
Hen	2.2 2.3
Pigeon	2.0
Pheasant	1.7
MAMMALS	
Ox	6.4
Man	6 0
Horse	5.8
Rat	5.7
Rabbit	5.3
Mouse	5.0

From

Indeed, the amount of substance suspected to be heredity determinant, should be the same in all cells of all the organs of the same species because all these cells should carry the same number of genes the amount should be twice lower in the spermatozoon, which presumably carries half of the heredity (the other half being supplied by the ovum) One would not have confidence in a substance which varied from one organ to another

By this indirect evidence, then, the heredity determinants should be DNA The evidence from an entirely dif

ferent direction was obtained in 1941 by Hollaender and Emmons (see also Zelle *et al*[14]) As will be discussed further on, ultraviolet irradiation induces mutations. Mutations by definition are replicable changes of the genes. Hollaender and Emmons determined which wavelength of ultraviolet light is most effective in inducing mutations, and compared it with the wavelength most effectively absorbed by each of the two suspect substances (proteins or nucleic acids) The answer was again unequivocal the wave length most effective in changing the genes was the one most effectively absorbed by nucleic acids and entirely different from the one absorbed by proteins. Thus, the nucleic acids again appear to be the genic material. Such an experiment does not tell us which of the two nucleic acids, RNA or DNA, is most likely to be the genic material but since we know that the genes are situated in the chromosomes, the main component of which is DNA rather than RNA it is again the DNA which seems to be the responsible agent.

In summary then all possible evidence indicates that the heredity determinants are special chemical substances, in particular all proofs indicate that these substances are nucleic acids, no evidence to the contrary or evidence for substances other than nucleic acids has ever been offered.

It would be of interest to see how the nucleic acids are distributed in nature (Table II) One notices first that there is no living entity down to the simplest viruses, which would lack this substance. It can be further seen that the two kinds of nucleic acid, the ribonucleic acid (RNA) and the deoxyribonucleic acid (DNA) are distributed very unevenly the smaller viruses, as a rule, contain only RNA, the larger-only DNA. As explained before, both are hered-

TABLE II

The Occurrence of Nucleic Acids in Nature

VIRUSES	
Tobacco mosaic	RNA
Poliomyelitis	RNA
Influenza	RNA
Rabbit papilloma	DNA
Vaccinia	DNA
Bacteriophage	DNA
FUNGI (including Bacteria) ALGAE	RNA and DNA
HIGHER PLANTS	RNA and DNA
PROTOZOA	RNA and DNA
HIGHER ANIMALS	RNA and DNA

ity determinants in their respective viruses. The *cellular* organisms, starting with bacteria, invariably contain both kinds of nucleic acids, though as mentioned previously only DNA seems in this case to be principal heredity determinant. It must be mentioned that the cellular ribonucleic acids, though belonging to the same group of chemical substances, may be quite different from the viral ribonucleic acids which are heredity determinants; in particular the latter seem to have a higher molecular weight, resembling in this respect the deoxyribonucleic acids, all of which seem to be heredity determinants.

The situation for the cells of higher organisms resembles that for the bacterial cell The amounts of DNA for individual species (regardless of organ) have been presented in Table I the amounts of RNA will vary from organ to organ

The distribution of the two kinds of nucleic acids (DNA and RNA) within the cell can be studied by cytochemical methods, using basic stains specific for one or for the other In another method, one can digest away RNA or DNA by means of enzymes known to be specific for one of them. Such enzymes are deoxyribonuclease (DNAase) which

specifically breaks down DNA and ribonuclease (RNAase) which specifically breaks down RNA. After digestion, any basic stain will reveal the location of the remaining (untouched) nucleic acid.

More quantitative is the method in which the tissues are homogenized and the individual cell components are separated from each other by differential centrifugation (centrifugation at various speeds). Each fraction can then be analyzed separately using convenient colorimetric methods*. The results of such an analysis for a typical cell

TABLE III
Nucleic Acid Content of Individual Components of Mouse Liver Cells*

Cell component	Micrograms phosphorus per 100 milligrams tissue RNA	DNA
Nucleus	10	23
Mitochondria	16	0
Microsomes	49	0
Non-particulate fraction	15	0

From [illegible]
F om

(mouse liver) are given in Table III. It will be seen that the ribonucleic acids are distributed throughout the cell and may conceivably serve for many different purposes; on the other hand, DNA is found only in the chromosomes, that is in the apparatus for the orderly transmission of heredity. This situation exists in all living cells which were examined thus far.†

It is worth while to spend sometime to see how the nucleic acids can "store" the genetic information and how this information can change in the process of mutation; this subject will be discussed in the next chapter.

* Such methods can of course be used also for whole cells (including bacterial cells).

† Except of course the cases in which DNA-containing virus or rickettsia has invaded the cytoplasm.

Chapter III

THE NUCLEIC ACIDS

Before we begin the discussion on the mode of action of nucleic acids, it is imperative to refresh our memory about the basic points of their chemistry The reader who is familiar with the general structure of nucleic acids may be tempted to bypass the first part of this chapter however it may be pointed out that new facts about this structure are constantly forthcoming in this book an attempt will be made to describe the structure as we know it at present

The molecules of nucleic acids are giant molecules of high complexity they can, however be broken by appropriate means, to simple building blocks called *nucleotides* Each such nucleotide is composed of one molecule of a *nitrogenous base* one molecule of a *sugar* and one molecule of *orthophosphoric acid* (Figures 3 4) There is nothing peculiar about this phosphoric acid but the sugar part is interesting The sugar is of the pentose type (5 carbons) † Two kinds of pentose sugars are found in nucleic acids one is D *ribose* which occurs in ribonucleic acids (RNA) (Figure 3) the other is 2-deoxy D-ribose, deoxyribose for short, which occurs in deoxyribose nucleic acids (DNA)

As of the beginning of 1959

† A six carbon sugar glucose, occurs in DNA of certain phage (in addition to pentose) but it is peculiarity

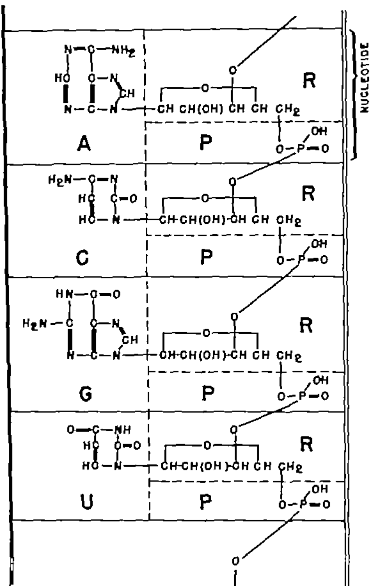


Fig. 3 Main components and structure of ribonucleic acid (RNA) A,C,G and U—nitrogenous bases adenine, cytosine, guanine and uracil. R—sugar D-ribose, P-orthophosphoric acid.

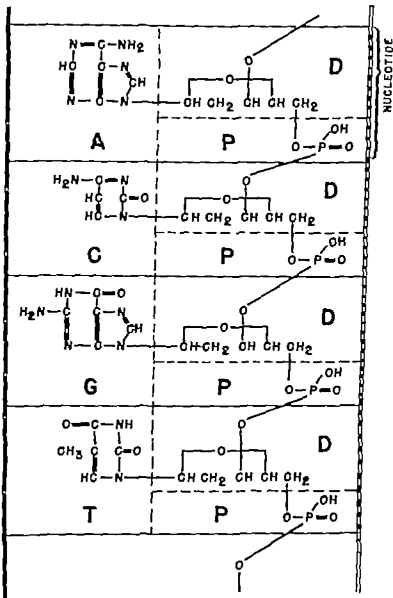


Fig. 4 Main components and structure of deoxyribonucleic acid (DNA) T— nitrogenous base thymine D—sugar 2-deoxy-D-ribose A,C,G and P as on Fig 3

(Figure 4)-hence the names of these nucleic acids. The deoxyribose differs from ribose only in that it has one less oxygen atom on the second carbon. This small difference must have very important implications in nature- however except for the fact that deoxyribose is a bit more reactive, we are at a loss to grasp why one sugar should be chosen for the nucleic acids of simpler viruses, and the other sugar for the nucleic acids of larger viruses and for the chromosomal nucleic acid of higher organisms.

The remaining components, the nitrogenous bases, are most interesting They can be classified into two categories the *purines* and the *pyrimidines* Each of the two nucleic acids contains, as major components, two kinds of purines and two kinds of pyrimidines, one for each nucleotide. These are- adenine and guanine (purines in both RNA and DNA) cytosine (a pyrimidine- in both RNA and DNA missing in DNA of certain bacteriophages)[14] uracil (a pyrimidine- in RNA only) thymine (a pyrimidine- mainly in DNA) 5-hydroxymethylcytosine (a pyrimidine- in place of cytosine in DNA of certain phages[5]) In addition to these main components, small amounts of various nitrogenous bases were recently reported in both RNA and DNA. These bases (not shown on Figures 3,4) are- 5-methylcytosine (a pyrimidine- in DNA of higher organisms only) 6-methylaminopurine (a purine- in microbial DNA and RNA)[6] 6-dimethylaminopurine (a purine in RNA) 2-methyladenine (a purine, in microbial RNA)[19] 2-methylguanine (a purine in microbial RNA) 1-methylguanine (a purine in microbial RNA) thymine (as a minor component in microbial RNA)[19]

As mentioned before, all these bases are located in their respective nucleotides, which bear corresponding names thus, a nucleotide composed of adenine, ribose and phos-

phoric acid is often called adenine ribotide· one composed of guanine, deoxyribose and phosphate—guanine deoxyribotide etc Other names used for these nucleotides are adenylic acid and deoxyguanylic acid respectively *Nucleoside* is the general name of a compound obtained by the combination of such a base and the sugar (or else obtained by the removal of phosphoric acid from a nucleotide) The corresponding names would be adenine riboside, guanine deoxyriboside etc

All these components are linked together in well defined ways, presumably always the same for all purines, and the same for all pyrimidines. All nucleotides, in turn, are linked together forming very long chains. Figures 3,4 give examples of such linkages. One notices that the phosphoric acid groups repeat themselves with striking regularity The distances *between these groups are always the same· 0 35 one* millionths of a millimeter it is exactly the distance between the individual amino acids in a protein chain. Thus, the two substances, nucleic acids and proteins are made to fit each other· nucleotides of one combining with the basic amino acids of the other forming nucleoprotein. Indeed, nucleic acids are never found in nature as such, but always as nucleoproteins from which nucleic acids themselves can be obtained only by removing the protein part (deproteinization)

It might be too boring for a non-chemist to study in detail all linkages in the DNA molecule. As a matter of fact it might have been already too boring to follow all these chemical names and formulas, so let us step right here and just look at the implications. The implications are that the structure of nucleic acid is very complex not less complex *than the structure of protein, which as we know* are capable of such an astonishing specificity like amino acids in

proteins, the bases in nucleic acids may be responsible for this specificity

What is actually the meaning of chemical specificity? And how can a molecule carry an "information"?

Consider a single nucleotide. By the virtue of its acidic group it will participate in some reactions, by the virtue of its basic groups it will participate in others. It may also catalyze certain reactions But the size and shape of the molecule and the distribution of electrical charges in it are such, that a nucleotide will by no means participate indiscriminately in all the reactions: the molecule is choosy it has a certain specificity In other words, by virtue of being itself the molecule carries certain chemical information, the instruction as to reactions in which it should participate (as a reagent or as a catalyst) and as to those in which it should behave indifferently

The amount of information carried by a single nucleotide is small it is such a small molecule. But if one links together a great number of them, then the individual small patterns of electrical charges are added and form a large, very complex pattern the molecule will now be very choosy indeed But there are only four different major nucleotides (two purine and two pyrimidine nucleotides) in each nucleic acid, and therefore they are repeated. If the four are repeated in a regular way always in the same order the pattern would become monotonous and no further gain in complexity or specificity would ever be possible. This is exactly the reason why the chemists for a long time disbelieved that the nucleic acids, especially DNA, could serve as a carrier of genetic information. they thought that the nucleic acids consist merely of repeating units called tetranucleotides, composed of one of each of the four nucleotides[1] Such a repetitious molecule would be very uninformative, indeed moreover all the molecules of DNA

of all the species would have to be the same and obviously could not serve as carriers of different hereditary characters. As will become clear further on, the sequence of nucleotides in nucleic acid is *very irregular* and the "tetranucleotide" unit simply does not exist but some thirteen years ago few would dare to doubt it, such was the authority of the author of the tetranucleotide theory a distinguished scientist P A. Levene. One chemist (Lehman Echternacht) even reported the actual isolation of this (purely fictitious) tetranucleotide[a] and seemingly the case was closed

Not quite. The tetranucleotide theory was simply based on a wrong chemical analysis, according to which the four nucleotides were present in equal amounts. In 1947 Gulland reported that the results of titration of nucleic acid somehow *did* not support the theory However Gulland's data were also wrong Chargaff and his collaborators undertook the accurate analysis of nucleic acids they found that the four nucleotides were present in very unequal amounts and therefore the tetranucleotide theory cannot be correct[a] The proportions of individual purines and pyrimidines were different in DNA of different species (Table IV) therefore, there must be not one, but many different nucleic acids. On the other hand, the DNA from different organs and different strains of the *same species* exhibit the same proportions of individual purines and pyrimidines (Table IV) These proportions are independent of the environment. Moreover as mentioned above, the sequence or distribution of individual purines and pyrimidines *in each nucleic acid is most irregular*[a] Now this situation is to be expected if various deoxyribonucleic acids are to be heredity determinants they *should be* different in different species but similar in different strains or different organs of the same species. The molecules of DNA

TABLE IV

The Proportions of Individual Purines and Pyrimidines in DNA of Several Species

Source	Moler Ratios Adenine / Guanine	Thymine / Cytosine	Purines / Pyrimidines
ANIMALS			
Man (sperm)	1.6	1.2	0.94
(thymus)	1.47	1.75	1.07
(liver)	1.42	1.80	1.09
Ox (sperm)	1.29	1.31	1.03†
(thymus)	1.29	1.43	1.1
Sheep (spleen)	1.26	1.36	1.01
Goat (sperm)	1.31	1.3	1.03†
Horse (spleen)	1.29	1.37	1.1
Rat (bone marrow)	1.34	1.39	1.00†
Hen (*erythrocytes*)	1.45	1.29	0.99
Turtle (erythocytes)	1.31	1.31	1.03
Salmon (sperm)	1.43	1.43	1.02
Herring (sperm)	1.25	1.32	1.00†
Trout (sperm)	1.32	1.36	1.1
Shad (testes)	1.3	1.43	1.01
Locust (whole)	1.43	1.41	0.99†
Sea Urchin *Echinocardium Cordatum*	1.93	1.79	1.00
HIGHER PLANTS			
Wheat (germ)	1.22	1.62	1.00†
LOWER PLANTS			
Saccharomyces cerevisiae (Baker yeast)	1.67	1.92	1.00
Escherichia coli	1.04	0.95	1.01
Serratia marcescens	0.76	0.63	0.92
Mycobacterium tuberculosis	0.4	0.4	1.1
Hemophilus influenzae	1.75	1.54	1.01
Streptococcus pneumoniae	1.45	1.75	1.0[illegible]
RICKETTSIAE			
Rickettsia prowazeki	2.09	2.07	1.12
ANIMAL VIRUSES			
Gypsy-moth polyhedral virus	0.72	0.72	1.07
Vaccinia virus	1.43	1.43	1.00
BACTERIAL VIRUSES			
E. [illegible] phage T	1.55	1.58	0.99

From [41]

† The data thus marked include 5-methylcytosine.

should be complex and irregular to be able to carry very specific information

The further we probe into the structure the more weird it appears: tracts of purines followed by tracts of pyrimidines may occur in certain sections complex irregularity in others. All these patterns are still meaningless to us we have not yet been granted the insight into the most valuable of nature's writings. But we know enough to suspect that this pattern or sequence is the very *code of heredity* the device to preserve genetic continuity If we calculate the number of possible patterns, just by changing the positions (sequences) of individual purines and pyrimidines in a molecule of DNA (of just one over all composition) we arrive at the figure of 10^{1} *** It is a large number certainly large enough to take care of all the genes which ever did or will exist † And all this was based on the assumption of four bases only As mentioned before, there are also present in DNA small quantities of additional bases, and these, like raisins in the cake, can add a lot of flavor to the specificity of DNA In addition one must not forget that our knowledge of the DNA molecule is actually in its infancy thus, many other features which add specificity could still be discovered

While the discussion above centered specifically around DNA, much of it can also be said about RNA. The molecule of RNA may be somewhat smaller but it is still sufficiently long for specificity It is just that we do not know as much about RNA as we know about DNA The reason for it becomes plain upon looking at Table III again. The

The distribution of amino acids in proteins is also most irregular so that the latter too, can carry very specific information, though not genetic information.

It can be easily calculated that the number of individuals which has ever existed in our species could not have been higher than $10^{[illegible]}$ and was probably lower than 10

DNA is situated in the chromosomes only it seems to have only one function and is considered to be one type of substance. On the other hand, the cellular ribonucleic acids seem to be everywhere- each in its own site may serve a different function and be a different substance Indeed, ribonucleic acids from different parts of the same cell were found to have different compositions (review in[illegible]) Such a situation was bound to slow down the research on cellular RNA. there just are not enough scientists to go around On the other hand, the heredity determining RNA of viruses are single substances, and considerable progress has been achieved especially in the study of the RNA of tobacco mosaic virus. Such RNA has giant molecules, though apparently not quite as giant as DNA. the latter may have a molecular weight of 6 million (about 20,000 nucleotides) the former 300,000[illegible] or 2 million The tetranucleotide" theory in RNA is just as invalid as in DNA, and the irregular distribution of individual purines and pyrimidines—just as bewildering There may be, however some difference in the spacial arrangement of the two nucleic acids.

While the scientists were enjoying the implications of the *irregularities* in the structure of nucleic acids, certain *regularities* were also found The most important of these is the fact that the ratio of all purines to all pyrimidines in any DNA is always equal to one. It is as if one purine always formed a pair with one pyrimidine This thought led to one of the most esthetic concepts in modern biochemistry the now famous model of the DNA molecule of Watson and Crick[illegible]

It was suspected for quite some time that the molecules of some proteins may have a spiral configuration, and that two or more such spirals could be wound around each other in space, like threads of a screw Now the distribution of atoms in a molecule can be measured by means of a spe

cal technique called x ray diffraction. On the basis of such measurements for purines and pyrimidines[illegible] Watson and Crick proposed that the DNA molecule is composed of *two complementary spiral strands* which are wound around each other (Figure 5) Each strand would actually be a

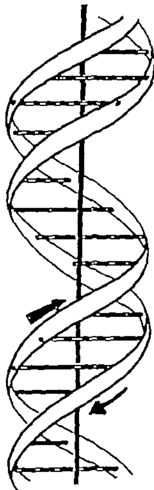


Fig. 5 Watson–Crick model of molecule of DNA The tw ribbons denote the two sugar-phosphate chains, and the horizontal bars—the pairs of bases holding the chains together (See Fig. 4)

string of nucleotides, as shown on Figure 4, except that it is a spiral in space and not a flat figure. The arrangement of the spiral is such that the phosphate bonds on each of the two spirals stick towards the outside, whereas the purines and pyrimidines-towards the inside, facing the complementary pyrimidines and purines of the other spiral. Here Watson and Crick utilized the previously found regularity (number of purines is equal to number of pyrimidines) the most logical model is such that a purine in one strand always faces a pyrimidine in the other The members of such a pair (purine-pyrimidine) are actually so close in space that their most proximal groups can form chemical bonds. These bonds are of the "hydrogen bond" variety they are not as strong as for example carbon to carbon bonds in chemical compounds, however since a molecule of DNA may have as many as 10,000 of the purine pyrimidine pairs, there are so many of these hydrogen bonds that they impart to the DNA molecule quite a remarkable stability As will be seen further on, some DNA molecules will withstand boiling for one hour few if any biologically active proteins would be that stable.

According to the calculations for distances in space, the parts do not fit together if a purine faces merely *any* pyrimidine it has to be always so that adenine faces thymine and guanine faces cytosine, or vice versa (Figure 6) Each of these is what is called a "complementary pair Now a moment of thought will convince us that this situation results in that the two strands are also complementary a strand with a specific sequence of individual purines and pyrimidines can pair only with a very specific strand that is, the one with the complementary (but not the same) sequence.

Thus, each strand determines the sequence in its partner each, then, must carry the entire information These

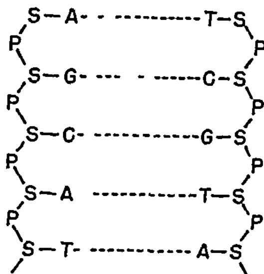


FIG. 6. Pairing of purines and pyrimidines in the Watson-Crick model A—adenine; G—guanine; C—cytosine T—thymine. S denotes sugar (deoxyribose) and P—phosphate. Solid lines—covalent bonds; broken lines—hydrogen bonds.

considerations led Watson and Crick to the second proposal of a mechanism whereby the molecule of DNA can replicate without losing or changing any of its information The mechanism is beautiful in its simplicity when ready to replicate, the two strands separate (by unwinding) and each rebuilds its missing partner Each strand can theoretically do it because each carries the full information consequently there will be two identical molecules where one existed before.

Now most scientists are artists at heart and they were moved by the logical esthetics of these models as a result, the acceptance of the models did not encounter the resistance which usually meets new and bold concepts. But some scientists recall the pitfalls in accepting a concept because of its simplicity the "tetranucleotide theory" can serve as

one example. The critics point out that the x-ray diffraction studies, on which the model is based, account at best for only half of the structure the other half like the other half of the moon, is invisible and conceivably can furnish some surprises such as short side chains, chemical linkages different from the accepted ones, etc. In particular it is still difficult to fit into the model some of those bases which are present in DNA in small quantities[1] By and large, however it is felt that with all the limitations in mind, the Watson and Crick model still fits the experimental data best for at least the essential part of the DNA molecule The original concept of the mechanism of replication (separation of threads) has stimulated considerable speculation as a result many essentially equivalent mechanism of replication have been proposed (review in) The basic concept of Watson and Crick has recently been supported by the results of the ingenious experiments of Meselson and Stahl These scientists grew bacteria on medium containing a heavy isotope of nitrogen (N) so that all the substances in the cell, including DNA, became labelled with heavy nitrogen. They then grew these bacteria for two generations on ordinary nitrogen (N) isolated the DNA and, by means of an ingenious new centrifugation method, determined how the heavy nitrogen was distributed among the DNA molecules. The result was that half of the molecules had some N label and the other half—only N A moment of thought will convince us that this result clearly supports the Watson and Crick model of DNA replication during the first division on N medium, the two strands of each molecule labelled with N became separated and each rebuilt the missing strand, using the only available nitrogen that is ordinary N each molecule

[1] Still the 1:1 ratio of purines to pyrimidines is valid for the entire molecule.

consisted of one old strand labelled with N and one new strand labelled with N^1 during the second division the strands separated again and the ones containing only N (half of all the strands) rebuilt the missing ones, also with N —consequently such complete molecules (half of all the existing ones) will contain only N

Thus, it is very likely that the individual strands do not break down during the replication and that Watson and Crick were correct about their suggestion as to how the molecules reproduce in the cell Well, obviously in the cell " After all, one cannot reproduce nucleic acid *in vitro* that would almost be the creation of new genes in the test tube, a science fiction stuff

One cannot? As in many other fields, science is catching up with science fiction and below is the report of such recent fascinating endeavors.

In 1955 Ochoa and Grunberg Manago were working in New York University on an enzyme polynucleotide phosphorylase which removes the second phosphate from nucleoside diphosphates and attaches a phosphate from the medium instead. Now this second phosphate in nucleosides is a very interesting one- it is linked to the first phosphate by a so-called "pyrophosphate" bond which is known to carry high energy thus, such a nucleoside diphosphate is capable of many reactions requiring energy such as synthetic reactions. When this reaction was performed under special conditions, the liquid became very viscous [1] and would not even flow out of a beaker turned upside down Evidently the enzyme in the beaker had synthesized a high molecular weight substance from the small fragments. The scientists realized the importance of this discovery and devoted their full effort to the elucidation of the phenomena involved It was soon found that one can obtain at will, a product which has only one kind of nucleoside (for ex

ample a poly adenylic acid) or a product which has two, three or all four this last substance was similar to a ribonucleic acid. The gross chemical composition, the kind of chemical bonds, the structure in space, even certain auxil iary biological properties, all were equivalent to those of natural RNA (reviews in) But, as will be discussed later on, the main biological rôle of cellular RNA is largely unknown, and so is a chemical method for finding the fine structure of RNA. In view of the lack of these one cannot be sure that the RNA like substance indeed has a proper sequence of nucleotides or carries the proper chemical in formation. What was worrying the scientists was that they could not put their finger on anything in this beaker which would tell the newly synthesized substance what this se quence or information should be. The synthesizing enzyme? No, this is unlikely because one enzyme is supposed to synthesize all kinds of molecules of RNA. Some sort of a starter (here called primer") which would make the new molecules into its own image? That would be most logical, it would also best fit the concept of RNA replication *in vitro* To date such a primer has not yet been found. Per haps it never will be perhaps the synthesis of RNA is more complex and less independent than one thought. It is, how ever of interest, to note that the small fragments of RNA, containing few specific nucleotides, were found to speed up the synthesis of larger fragments containing these spe cific nucleotides, thus serving as a kind of simplified primer

While this was going on in the field of RNA studies, a group in St. Louis under the leadership of Kornberg discovered another enzyme which synthesizes DNA *in vitro* (review in) The enzyme, first found in bacteria (*Escherichia coli*) but recently reported to be present also in mam-

consisted of one old strand labelled with N and one new strand labelled with N ^{4} during the second division the strands separated again and the ones containing only N (half of all the strands) rebuilt the missing ones, also with N —consequently such complete molecules (half of all the existing ones) will contain only N

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mals seems to work on a different principle than the enzyme which synthesizes RNA For one thing the starting materials for DNA were the deoxyribonucleotides carrying two, rather than one, extra high energy phosphate groups. For another thing the synthesis would go only if all four deoxyribonucleotides were present indicating that the enzyme has indeed all intentions to make only a good DNA. But the main feature was that the enzyme really needed a primer a pre-existing molecule of DNA Once such is present, the synthesis goes ahead, and the newly made DNA (up to twenty times more than originally put in) has the same base composition as the primer[44] The origin of the enzyme does not matter the enzyme from bacterium *Escherichia coli* given human DNA as a primer will synthesize a product of the base composition of human DNA. The proportion of the individual nucleotides in the mixture does not matter either the product always follows the primer

One now asks again does the product possess also the fine composition (the genetic information) of the natural DNA given as primer? To answer this question one should use a primer having some biological activity (such as the transforming principle) and then test whether the synthetic product also has this activity To date such an experiment could not be performed because the preparation of the synthesizing enzyme still contained as impurities some deoxyribonuclease, an enzyme which causes rapid destruction of the transforming principle (see below) When the experiment will be finally performed it may still turn out that all the enzyme can synthesize is an artifact devoid of genetic information (so called nonsense DNA") Should, however the synthesis of the active transforming principle be attained, then we would be witness-

ing a true reproduction of naked genes in a test tube, a fantastic, perhaps frightening achievement.

In the meantime, organic chemists are striving to synthesize fragments of nucleic acid in a purely chemical way without the enzymes. Various starting materials (nucleotides, both ribo- and deoxyribo-) can now be synthesized at will, due to methods developed mainly by Todd (Nobel Prize 1957). Using these building blocks, Michelson has succeeded in synthesizing fragments of RNA, and Khorana —fragments of DNA, by pure organic synthesis, without enzymes or primers. Moreover the composition and even the *sequence* of nucleotides can be controlled at will. These fragments, however are rarely longer than 15 nucleotides; natural DNA may have as many as 10,000 in one strand, so it is still a long way to go. Furthermore, although the fragments are most useful for many purposes, *the synthesis of a nucleic acid carrying genetic* information is unlikely to succeed in this way in the absence of a primer who is going to tell the chemists which sequence to choose, out of 10[illegible] possible ones?

In all our discussions we have used the word molecules, in the plural form this, however should not imply that the molecules of DNA, from one cell for example, are all alike. How could they be? After all, they must represent different hereditary characters. The scientists long felt that such molecules indeed must be different perhaps *each* molecule of DNA in the cell is different. The experimental evidence for this belief was furnished again by the transforming phenomenon (see previous chapter) in transformation as a rule only one hereditary character at a time is transferred, as if each character were indeed situated in a different molecule thus, the molecules must be different. The direct proof that they are different has been furnished by Chargaff *et al.* and others. It was found that by spe

cal methods one can separate the DNA (from one type of cells) into separate *fractions* which differ in their base composition Thus, the compositions given in Table IV or indeed any other chemical or physical properties reported for DNA, are always merely the *average* properties.

Now it would be very nice if every gene" would be *located in a different molecule of DNA but one might* ask an embarrassing question are there enough DNA molecules in a cell to go around? The answer is in many cases not nearly enough. Take for example a cell of bacterium *Hemophilus influenzae* it is a small cell, but nevertheless very much alive. Now one is accustomed to think that there are millions of molecules of every substance within the cell yet this cell has only 200 molecules of DNA * That is all Clearly a living cell must have more than 200 genes or characters. Perhaps, then, there is more than one gene to a molecule of DNA? Recent evidence indicates that this indeed may be so Upon closer examination, it was found that in some transformation experiments two characters may be transferred together as if they were linked in one molecule of DNA[66] In this way cells were obtained which never existed in nature monsters having two capsules in place of one In another case, the linkage of two characters has been obtained as a normal feature and in recent years numerous reports indicate that this is indeed a rather common phenomenon. Just how many heredity determinants can find a place on one molecule of DNA? Recent speculations, based on the determination of the vulnerable segment in a molecule, indicate that one can divide the molecule into 6 to 10 parts and such parts will still be active[11,12] But from the field of bacteriophage studies comes another approach Benzer[8] has performed a pains-

* A larger bacterial cell, of *Escherichia coli*, has about 1000 molecules of DNA, human haploid cell has about 150,000.

taking study of crossing-over in phage (a process akin to crossing-over in the chromosomes of higher organisms) and came to the conclusion that on that basis a unit of heredity (strictly speaking a unit of crossing-over) would be no bigger than *a few* nucleotides, if so there would be thousands of such units per *one* molecule of DNA. In any case, the feeling is that the old notion of one gene per molecule is definitely outmoded.

Closely connected with the concept of the "size of the marker" is the problem of just how much one can injure the molecule of DNA without producing its inactivation or change. The problem is important: it has bearing on the phenomenon of killing by radiation and the process of mutations.

Before we can embark on the problem of injury to DNA we must decide what we want to call the uninjured or "native" DNA.

At present it is not known whether an even most carefully isolated DNA preparation can in all respects be identical with the DNA as it existed in the living cell: the DNA is never found in the cell as such but merely as part of a nucleoprotein, which in turn, may have bonds to still other substances. The term "native" when applied to the preparation of DNA *in vitro* is therefore rather misleading: what this usually means is that such a preparation exhibits certain features which it must have had when it was in the living cell. The most obvious and the most important of these features is the proper biological activity. It is indeed a feature of which one can safely say could not have been created artificially by the process of isolation. Such an active DNA preparation may be called "*functionally intact*" and can serve as a yardstick, obviously its properties are more constant and are of *more* interest than the properties of the products of an undetermined degree of denatura

tion" in particular the studies of the functionally intact DNA preparation may lead to correlation between the function and the structure.

As discussed before, the transforming activity is such a proper biological activity which can be demonstrated in certain bacterial DNA preparations: these can therefore be called functionally intact Having this yardstick we can now subject the DNA preparations to various treatments and see what these treatments do to the biological activity and what they do to the physical and chemical properties of DNA One conspicuous property of DNA in solution is its high viscosity this property is common for all highly *asymmetric* particles. By "asymmetric" it is meant that the molecule is much longer than it is wide, and DNA is a typical example It has been mentioned that DNA has a high molecular weight (about 6 million) because it is composed of so many nucleotides, a compound of this sort is called a high polymer As stated previously these nucleotides are arranged linearly (see Figure 5) in the form of two long intertwined threads, so that each molecule is about 8/10,000 of a millimeter long though only 1/400 of that in diameter· indeed, a highly asymmetrical structure when fully extended. If we now heat the molecule above 180 F for one hour or so the molecule will collapse and become less asymmetrical therefore, the solution will be less viscous. At the same moment, the biological activity will decrease, and this serves as one of the indications that the biological activity is indeed vested in the *highly polymerized* uninjured DNA Other injuries can be caused by acid (below pH 5) or alkali (above pH 10) acid, for example, can break some of the phosphate bonds or split off purines this will make the thread shorter and devoid of original information"—again the loss of viscosity and the loss of biological activity start at the same moment De

oxyribonuclease (DNAase) the enzyme which breaks ("depolymerizes") DNA, and DNA only is even more harmful. Strong DNAases are present in pancreatic juice and in the secretion of certain hemolytic streptococci, but specific DNAases are present in every cell though they may be inhibited so as not to cause too much damage when the cell is still alive Now if one confronts DNA with as little as one millionth of its weight of pancreatic DNAase, the latter will break enough phosphate bonds so as to completely destroy all the biological activity within 20 minutes the decrease in viscosity follows later on. The action of this specific enzyme was one of the original proofs that the transforming principle is indeed DNA.

Many other agents can readily destroy DNA Among them, the most interesting are those which are also known to be *mutagenic agents* This subject will be discussed in the next chapter

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As discussed in the previous chapters, the only chemical substances known at present to fit the rôle of heredity determinants are nucleic acids. One should not exclude the possibility that some other substance such as protein might some day be discovered to also possess the properties of heredity determinants. But right now we know only about nucleic acids and that is all we can discuss. We know for instance, that if we infect the cell with nucleic acid from a mutant virus, it will reproduce in the cell as nucleic acid of a mutant virus. If we transform the bacterial cell with DNA from a mutant cell, such DNA will reproduce as the one from the mutant cell in other words, the *mutated DNA* will reproduce as mutated DNA. Now what is a mutated nucleic acid? What change in what feature of the structure of nucleic acid is retained upon reproduction? In other words, what is the chemical basis of mutation? The answer is we do not know but we can make some intelligent guesses. As mentioned in Chapter III one guess is that the specificity of nucleic acids may reside chiefly in the sequence of various nucleotides. If the situation were really as simple as this, then mutation would be just a change in the sequence of nucleotides. It would be difficult to visualize how such a change could come about, unless it occurs as a *fault* during the replication of nucleic acid. Indeed, evidence has often been presented that mutations can occur only during cell division (i.e., during the synthesis of nucleic acids) But evidence to the contrary has been reported as well. in *Drosophila* old spermatozoa include more mutants than the young ones in bacteria, a certain proportion of mutations also occurs during the resting (stationary) stage (Ryan *et al.*[¹⁴]) It appears that the decision for a mutational change can somehow be registered in the "resting" molecule of nucleic acid though the actual execution of the "change order" occurs later at the time of

cell division. What could possibly be the form in which such a decision to mutate is imprinted upon the molecule?

It has been observed that in many cases a delay exists between the moment of action of mutagens and the actual production of a mutant in certain instances the prospective mutants can still recover during this time

All these observations taken together have led several scientists to suggest that perhaps all the mutagens do is to bring the gene to an *unstable* state, from which the gene can either revert back to normal or turn into a mutated gene this latter process could take place even in the absence of the mutagen (for a review see [7]) Such a hypothesis would also explain why mutagens which exert upon the gene an entirely different influence (such as irradiation and mustard gas) may result in production of the same kind of mutant.

Unstable genes that is genes which have high spontaneous mutation rates, have been found in nature in many organisms (*Drosophila* maize bacteria) The strains carrying such genes are not necessarily freaks they may survive and even accelerate evolution [11] Unstable genes can also be produced in the laboratory (bacteria) by treatments *in vivo* similar to those which will produce unstable DNA *in vitro* perhaps we are dealing with the same phenomenon The chemical nature of the injury involved is not clear some bonds must be broken which renders the molecule less resistant or less rigid, though still active If the injury is not repaired upon reproduction, a perpetually unstable strain may result.

Turning now to the discussion of the mutagenic agents themselves, one stumbles upon the textbook division of mutations into two classes. spontaneous and induced A modern scientist who does not believe in miracles, must assume *that the spontaneous mutations are also induced by muta*

gens but the latter are largely unknown and are with us all the time. The problem is of utmost importance the phenomena involved may govern the spontaneous incidence of some types of cancer and some hereditary diseases, the spontaneous appearance of resistance to antibiotics in microorganisms, and last, but not least, evolution. Accordingly the scientists have given considerable attention to the question of what these unknown agents could be Perhaps the oldest and still respected hypothesis is that the culprits are the thermal oscillations which of course are always with us such oscillations on rare occasions may inflict an injury (or cause faulty reproduction) in a large molecule of the gene Another suspected causes were natural radiations (cosmic rays, radioactive elements in the earth) which are known to induce mutations and are always with us. However it has been calculated that the level of such radiations was* too low to account for all spontaneous mutations.

Then, there are chemical mutagens to consider The known chemical mutagens are discussed below (under "induced mutations") but there may be many unknown ones within the cell the naturally occurring adenine[1] and vitamin C (ascorbic acid) have also been reported to induce mutations. These two substances are necessary for life- this may automatically mean that we cannot live without mutations and evolution.

Some of the recorded spontaneous mutation rates for a few representative organisms are listed in Table V It can be seen that, in general, mutation is a rare phenomenon, by and large, heredity proceeds without disturbances. Some species, the famous African fish coelecanth, reptile tuatara, and even the ordinary opossum and cockroach, are reported to look the same today as they did over 50 million years ago- the mutation rates in these species may be quite low

* Before the atomic age

TABLE V
EXAMPLES OF SPONTANEOUS MUTATION RATES

Species	*Gene*	*Mutation Rate*	*Reference*
Bacteriophage	Host range	10^{-}[illegible]	10
Escherichia coli			
Stable strains	Streptomycin resistance	10^{-}[illegible]	108
	Streptomycin dependence	10^{-1}	108
	Phage resistance	10^{-}[illegible] 10^{-}[illegible]	108
	Penicillin resistance	10^{-}[illegible]	108
	Lactose non-fermentation	10^{-}[illegible] 10^{-}[illegible]	102
	Histidine dependence	10^{-}[illegible]	108
Unstable strains	Lactose non-fermentation	2 10^{-}[illegible]	101
	Histidine dependence	10^{-}[illegible]	100
	Reversion to full size colony	10^{-}[illegible]	126
Zea mays (corn)	Shrunken seeds	10^{-}[illegible]	10
	Purple color	10^{-}[illegible]	10
Drosophila melanogaster	Average lethal gene in the second chromosome	10^{-}[illegible]	10
Man	Epiloia	10^{-}[illegible]	10, 109
	Retinoblastoma	1 4 10^{-}[illegible]	10, 109
	Albinism	2.8 10^{-}[illegible]	10, 109
	Congenital total color blindness	2 8 10^{-}[illegible]	10, 109
	Microcephaly	3.0 10^{-}[illegible]	10, 109
	Hemophilia	3 2 10^{-}[illegible]	10, 109

indeed. But, in general, one cannot say that a given spontaneous mutability is a characteristic of a species; as mentioned previously (see also Table V) unstable genes may exist even in otherwise stable species. In extreme cases studied in *Drosophila* up to [illegible]5 *per cent* of the progeny are mutants[?] This really looks like a break-down of the orderly process of transmission of heredity Such phenomena have been called *mass mutations* they occur rarely and thus far have been reported for single genes only otherwise the organism probably could not survive. But in less extreme cases, the increased spontaneous mutability of several though not all genes in the same cell has been found

1963 Li What is the chemical basis of it? Some less stable DNA molecules? A selective mutagen in the cell? The writer of this book (who, incidentally is biased) thinks that this is a very important problem.

The *induced* mutations are produced by subjecting the genetic material to a variety of known agents (mutagens) the comparatively high mutation rates so obtained are superimposed on the rather low spontaneous mutation rates. Some of the typical strong mutagens are listed in Table VI The table also lists the possible effect the agent could have on DNA

The most pronounced havoc among the genes seems to be due to the action of little gremlins called *free radicals*

TABLE VI

EXAMPLES OF STRONG MUTAGENIC AGENTS

Name	*Possible Effect on DNA*	*Inactivation of DNA Having Transforming Activity*
RADIATION Ultraviolet, X-rays	Largely unknown probably caused by free radicals produced by the agent	Strong
HEAT†	Breaking of H-bonds, collapsing the molecule	Strong
SELF-OXIDIZING AGENTS Ferrous ion, Ascorbic acid	Largely unknown; probably caused by free radicals produced by the agent	Strong
MANGANOUS ION	Unknown	None
ALKYLATING AND ESTERIFYING AGENTS Sulfur and nitrogen mustards, Dimethyl- and diethyl sulfate, methyl iodide, β-propiolactone, etc.	Alkylation, probably on 7-th nitrogen of guanine and adenine; esterification of phosphoric acid‡	Strong

From. † From ‡ From

The free radicals can be described as very short lived and therefore very reactive chemical fragments (such as HO, or OH (*not* a OH ion) They are produced by the action of radiation on water by self-oxidizing substances (such a ferrous ions, ascorbic acid etc.) and by many other processes. The chemical nature of the action of free radicals on DNA in a living cell is still not clear despite the voluminous literature on this subject

The action of mustards and other so-called alkylating agents is better known. These agents attach their alkyl group to the purines (but not pyrimidines) of DNA on the nitrogen in position 7 causing this nitrogen to assume a positive change. The agents also produce esterification of the phosphate of DNA Both these effects are likely to cause disturbance (unstabilization?) of the DNA molecule we do not know as yet which of the two is more harmful.

It is probably easier to destroy a gene than to change it while still active It has been suggested that the processes leading to mutation and to death, caused by mutagenic agents, are essentially the same, with the exception that the latter is accompanied by more extensive molecular changes. If this is indeed so, then the *inactivation* of DNA by these agents could be a demonstration of a "too strong mutation" (mutation which is lethal to the DNA) In Table VI the inactivation of DNA (transforming principle) has also been indicated. It will be seen that, in general, the transforming principle is sensitive to all strong mutagens. This fact may suggest again that the DNA is indeed the site of their mutagenic action.

It has also been found that the individual genes in the same preparation of the transforming principle (DNA) may have different stabilities towards mutagenic agents[31] Thus, if a cell is killed by irradiation, some of the genes are

inactive whereas others may remain active. The physico-chemical nature of this difference is still unknown the difference in length of the individual molecules of DNA has been indicated as one of the factors.

Inasmuch as mutations are seriously considered to be one of the causes of some types of cancer it is of interest to discuss the *carcinogenic agents* from the point of view of their action as mutagenic agents in general, and of their reaction with DNA in particular These agents can be arbitrarily divided into two classes 1) those which are unquestionable strong mutagens (e.g radiation and mustards) react strongly with DNA and even in minute doses inactivate the transforming principle and 2) those which are weak or questionable mutagens (e.g carcinogenic hydrocarbons) do not react with DNA and have no effect upon the transforming principle. While the first class fits nicely to the theme "cancer—mutation—DNA, the second class does not, and the speculations concerning this failure have not been very fruitful.

Closely connected is the subject of *carcinostatic agents* i.e the agents which check the growth of the cancer cells. Theoretically any agent which interferes with the life of the cancerous cell is, in a way also a carcinostatic agent. The choice is narrowed, however by the requirement that the agent does not interfere seriously with the life of the normal cells. Among many agents in this now very active field of *chemotherapy of cancer* the favored ones are those which interfere with the DNA of the cell Here, too, the agents can be arbitrarily divided into several classes.

To the first class belong agents (e.g., various mustards) which are known to be mutagenic (review in) react strongly with DNA and inactivate the transforming principle in fact it has been shown that within one group of

mustards the order of their potency against one type cancer (Walker carcinoma) corresponds closely to the order of their potency to inactivate the transforming principle[1] One could speculate that the action of the carcinostatic drugs of this class is to make the DNA of cancer cells unfit for reproduction. Obviously this is still far from proven

The second class includes chemicals (e.g., analogues of natural substances) which interfere with the synthesis of essential cell constituents. Some of them are known to interfere with the synthesis of DNA or RNA but in general the chemicals in this class are not strong mutagens, they do not react with the nucleic acid already made, and do not inactivate the transforming principle. The third class includes empirical drugs whose chemical action is still largely unknown.

An old dream of geneticists is to induce "directed mutations this would lead without waste to mutants with desirable features, and in time—to new species, perhaps even to the next human species. But, although in a few cases the type of chemical reaction of the mutagen with DNA can be estimated, the actual outcome of the mutation is largely unpredictable Some genes are more sensitive to one mutagen, different genes—to another still, one mutagen may produce all sorts of mutants. This is understandable when one realizes that the choice of which molecules and which spots on the molecule of the gene are to react are largely beyond our control Mention has been made of different stabilities of individual transforming principles thus, *in vitro* specified genes may become deliberately *destroyed* whereas others remain active However the corresponding *mutation in vitro* has not yet been achieved As to the experiments *in vivo* a suggestion has been made to make the mutagenic action more specific by focusing the ultraviolet rays on a particular spot of the chromosome[1,6] [1] however

such methods are still rather crude. Perhaps most resembling directed mutations are certain types of transformations known under the name of "allogenic"[71] the transformed cells exhibit a feature *different* from the one carried by the transforming principle applied. It is as if the transforming principle, (DNA) upon entering the host cell reacted with the DNA of the cell to produce a new DNA. The case of "linked" characters" mentioned in the previous chapter serves as a typical example the DNA determining the capsule of type *b* (DNA_b) enters the cell carrying DNA to determine capsule *a* (DNA_a) The result is a cell with two capsules, *a* and *b*- such cell now contains a new DNA (DNA_{ab}) which carries both features (*a* and *b*) presumably in one molecule. The results of such experiments are largely predictable—hence classification as "directed mutations." This change ($DNA_b + DNA_a \rightarrow DNA_{ab}$) cannot be accomplished by merely mixing the two DNA in a test tube only the cell can do it. For this reason many scientists shudder to call it a mutation they prefer to use the term "crossing-over within a molecule." Perhaps there is no sharp dividing line between these two terms.

In recent years, an entirely different kind of change in nucleic acid molecules became feasible. Biochemists have been experimenting for quite some time with analogues, that is substances which look like the natural ones but not quite: they may have, for instance, a wrong chemical group in a right place of the molecule, or a right group in a wrong place. As mentioned in the discussion of carcinostatic

It may or may not be out of context to make mention about another project which could become feasible as soon as the micrurgical technique becomes sufficiently refined. The project is to transplant an entire chromosome or its fragment from one nucleus to another If the fragment is long enough to include the spindle attachment, the transplant should "take (in somatic cells); this would produce directed" hybrid-like cell not obtainable by other means. The transplantation of another nuclear particle, the nucleolus, has already been achieved.

agents, such analogues, when properly designed, inhibit the growth of the cell, largely because they compete with the natural substances One group of such analogues, designed by Hitchings and Elion has a halogen (bromine, chlorine, iodine or fluorine) in place of methyl (i.e. on carbon number five) *of thymine, which is an essential component* of DNA (Figure 4) When bacterial strains which needed thymine to grow were cultivated in the presence of such analogues (especially one called 5 bromouracil) the DNA of the cells was found to contain up to 50 percent of 5-bromouracil in place of thymine [1-11] It was as if the cells mistook 5-bromouracil for thymine, because of their resemblance indeed, of all these halogenated analogues, the one best resembling thymine in size (5-bromouracil) was *also the one which fooled the cell most easily* Moreover bacteriophage grown on such cells also contained 5 bromouracil in place of thymine Such cells or bacteriophage particles remained alive moreover they now contained a high proportion of mutants which persisted even when the organisms were subsequently grown on normal media [16, 17] 5 Bromouracil is apparently quite selective in that it likes to induce some mutations more than others [5] still, a large number of genes is affected

Recently *the incorporation of other analogues into bac*terial DNA and into bacterial and viral RNA [18] has also been reported

Thus, one can now induce the cell to make unnatural genes yet, the "directed mutations" are almost as remote as ever To obtain advantageous mutations one still has to resort to a wasteful procedure of inducing many and select the few desired all one can do is increase mutation frequency by the use of strong mutagens or unstable genes, the former procedure *(strong radiation) is now used on* plants in Brookhaven National Laboratory

Chapter 3

INTERMEDIARY SYSTEM AND CYTOPLASMIC INHERITANCE

As DISCUSSED in Chapter I the intermediary system carries the orders of the genes to the site of the final peripheral reactions. It is obvious that this system itself is also under gene control and can itself be considered an interesting object of study.

About this intermediary system we know very little. The information carried by the system is very complex and therefore one suspects that the system itself is complex. If the information is carried by substances, they must be fairly complex, too: proteins, nucleic acids or both would fit this rôle. If it is nucleic acid, then it must be RNA because DNA as a rule is not found outside of the nucleus. There are reports that something visible comes out of the nuclear membrane into the cytoplasm [22]; there are other reports that RNA passes from the nucleus to the cytoplasm [23]. But the evidence catching the DNA in the act of handing the orders to any other substance (RNA?) is still non-existant. Instead, in recent years more attention has been devoted to the other end of the line: the RNA handing the orders to the proteins. Evidence is, indeed, accumulating to indicate that the RNA is essential for the synthesis of proteins and intelligent guesses are made as to how the former determines the structure of the latter. It was observed long ago that cells

which do not produce proteins (such as spermatozoa) contain very little RNA, whereas those whose main function is to produce protein (e.g., glands) contain large amounts of RNA, moreover the gland cells which produce protein intermittently contain more RNA during the period of protein synthesis than during the remaining period. An inspection of Table III reveals also that those parts of the cell which are known to be concerned with active protein synthesis (microsomes) contain more RNA than any other parts of the cell. Recent experiments have shown that the synthesis of protein stops short if the RNA is removed by extraction, destroyed by enzyme ribonuclease or poisoned by analogues (recent review in[12]) upon the addition of RNA, the synthesis is resumed. It appears now that the amino acids which became "activated" by a special process, can attach themselves to smaller molecules of RNA or RNA fragments[19-] and eventually give rise to a protein, it is there that the information residing in the sequence of nucleotides of RNA is believed to be translated into the information residing in the sequence of amino acids of the protein. The reverse process, the synthesis of RNA can also take place in this system The whole situation is still far from clear but at least a beginning seems to have been made.

Since the site of the intermediary system is in many cases in the cytoplasm, it is fitting to discuss here the problem of *cytoplasmic inheritance*

From the previous discussion one may get an impression that in the cell the self-determining particles, the genes, must reside exclusively in the nucleus. This impression results from the study of the precise mechanism of cell division which assumes that each daughter cell receives one of each highly specialized nuclear particle, the chromosome. No mechanism assuming an even distribution of cy

toplasmic particles upon cell division has ever been discovered thus, one argues, the cytoplasm cannot determine heredity because upon each division the daughter cells would be unequally endowed with heredity and evolution would be a mess. But, if one comes to think of it, this for higher organisms has never been adequately proven. True, many genes are known beyond any *doubt to be* carried by chromosomes. But every higher organism inherits the entire cytoplasm of the egg is this and other cytoplasms entirely devoid of genes?

The problem of the genes in the cytoplasm, the so called *cytogenes or plasmagenes* occupied the scientists for a long time. The problem goes back to the discovery of a rat an rare phenomenon of *maternal inheritance* the inheritance of certain features from the mother and not from the father The case of inheritance of the direction of shell coiling in pond snails is a classical example (review in) Could there not be a great number of such features which we inherit in the egg from the mother only such as the enzymatic *starting* dowry which determines the embryonic development, making us man and not rat? Systems which are hidden because they are so obvious that we take them for granted?

There could be. But the evidence to date either refutes this notion or else reveals that only few features are carried by cytoplasmic genes besides, even these are not independent but are controlled by nuclear genes. In the case of inheritance of the direction of coiling in snails, the feature is inherited from the mother however it is determined by normal chromosomal genes. It just so happens that the information is laid down even before the egg is fertilized so that the contribution of the father will be felt only in the next generation. The best case for cytoplasmic inheritance would be if one could find in the cytoplasm some sort of

self-determining self reproducing particles, like those in the chromosomes. For a while it was thought that such were found in the case of the Kappa particle in the protozoon *Paramecium* (review in [?]) those cells which are "infected" by these mysterious particles are able to produce a substance that can kill some other cells. The particles were ultimately under the influence of the chromosomal genes, still, the particles could be called self-reproducing because they were not lost after many cell divisions and because the nuclear genes could not make them unless at least one particle was present. However it was soon discovered that these cytoplasmic particles contained DNA and were actually rickettsia like invaders. The proponents of the cytoplasmic inheritance maintain that it is merely a matter of semantics whether the particles are called an invader (which implies a different species, rickettsia or virus) or a non-obligatory component of the cell [?] perhaps some day a comparison between the DNA composition of the particles and the host will settle the argument

The phenomenon of the maternal inheritance of sensitivity to carbon dioxide in *Drosophila* also belongs to this category such inheritance was found to be due to a virus carried by the egg cytoplasm. But the cases of inheritance of the plastids in plants (review in [?]) cannot be shaken off so easily Plastids *are* integral parts of the plant cells although under nuclear control, plastids are endowed with the power of self reproduction in the cytoplasm the hereditary defects in plastids are transmitted almost exclusively in the maternal line since as a rule the male pollen does not carry them. Many cytoplasmic particles contain RNA, it is tempting to speculate that this RNA is the self reproducing cytoplasmic information-carrier

Perhaps the most interesting experiments are those that involve transplantation of nuclei in amoeba (review in)

and hybridization in certain plants [2, 43, 1] the maternal" cytoplasm changed somewhat the information carried by the paternal" nucleus and continued to do so for countless generations since there is no sign of dilution of this maternal information, some self reproducing cytoplasmic particles can be suspected again.

The concept of *cytoplasmic mutants* has also been introduced. The cytoplasmic mutants would differ from the normal nuclear or chromosomal ones in that the former are not subject to Mendelian segregation. Actual cases have been reported by Ephrussi in yeast [44] and by Mitchell in the mold *Neurospora* In the case of yeast it was found that the cells which normally form large colonies on an appropriate agar medium, in about 1 per cent of the cases may form only small colonies (petite) This growth inhibition was eventually traced to the lack of cytochromes *a* and *b* in such a petite strain. The petites always reproduced as petites" some sort of hereditary process was obviously involved. But it was not an ordinary nuclear heredity For one thing the mutants did not obey Mendel s laws: they did not segregate in expected ratios in subsequent generations. For another thing the mutations were completely irreversible—a rare phenomenon indicating the complete loss of some factor By studying the pedigrees of normal and petites, Ephrussi came to the conclusion that one deals here with self reproducing *cytoplasmic particles* whose presence determine the normal production of cytochromes. The particles can become irreversibly lost by a cytoplasmic mutation which can be either spontaneous or induced by a *cytoplasmic mutagen* (such as acriflavine) in both cases a petite strain results. One must stress that the cytochrome production, like everything else, is also under the control of ordinary nuclear genes; in fact, one can

The cytochrome system is essential for the respiration of the cell.

get "petite" by a normal reversible mutation of the nuclear genes. However the cells which have nuclear genes to produce normal cytochromes, are unable to do so unless at least one self-reproducing cytoplasmic particle is present An analogous situation exists in *Neurospora* (cytoplasmic mutant "poky" which also lacks cytochromes *a* and *b*)

In summary inheritance by self reproducing cytoplasmic particles exists beyond doubt. Why then, does one suspect that most essential hereditary information cannot be carried by these particles? The "petite" furnishes a partial answer: if the cell divides much faster than the particles are reproducing some of the daughter cells may end up having no particles—a defective "petite" results[1] this, as discussed above, is due to the fact that the cytoplasm lacks the apparatus for the precise transmission of heredity (chromosomes) One suspects that heredity as a whole could not be as orderly as it is if it depended mostly on the unreliable cytoplasmic inheritance. But, obviously much more research is needed to transform this suspicion into scientific truth.

Chapter VI

THE PERIPHERAL REACTIONS

In the previous chapters we have discussed the first system, the heredity determinants, which issue the "orders," and the second, intermediary system which carries these orders to the given location in the cell (Figure 7). We mentioned that certain particles in this second system may enjoy some limited local autonomy: they may self reproduce (cytoplasmic inheritance) while constantly under the

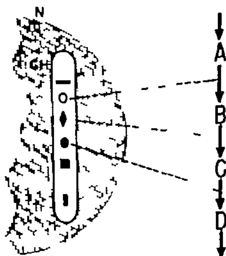


FIG 7 Transfer of genetic information to the peripheral system N—nucleus; CH—chromosome (See text for explanation.)

watchful eye of the nuclear heredity determinants. In this chapter we shall discuss the third system the peripheral reactions which are the final effect of these order-giving" and order-carrying events.

As mentioned in Chapter I, the scope of this subject is almost as vast as the entire biochemistry of the cell, for ev erything *in the cell is the result of heredity* In this chapter we shall only mention a few characteristic examples.

The general scheme of typical events is represented schematically in Figure 7 The third system, may for instance, consist of a sequence of reactions to change substance A to B to C and to D the final product D is either utilized by the cell, or secreted, as a glandular secretion, a waste etc As a rule, each of these reactions can occur only if a specific enzyme is present thus, one (favored) way the gene can control every step in the peripheral system is to allow *limit or prohibit the production of the particular* enzyme. It is not the only way of course. Instead of en zymes, the change may effect the co-factors, so called *coenzymes* which are the same for several enzymes, or the general conditions such as pH Entire sets of conditions may be changed by a single gene mutation and thus affect a great many peripheral reactions at once. To this belong gross morphological changes, *the blocking of permeability* of the cell membrane towards nutrients or excretion prod ucts etc. A bacterium *Escherichia coli* serves as an example of the latter block it *may* possess the enzyme to utilize cit rate as the only carbon source but as a rule it cannot do so because the bacterial membrane is impermeable to citrate (review in) it *does* possess the enzyme lactase to fer ment lactose but certain strains cannot continue the fer mentation because the bacterial membrane is impermeable to lactose. Eventually all the changes of morphology or permeability will be probably traced to changes in enzy

matic activity however for the time being we cannot study them as conveniently as we can the simple enzymatic reactions. Let us then go back to Figure 7

Figure 7 depicts also chromosomal genes affecting the individual enzymatic reactions. For simplicity they are arranged in the chromosome in the same sequence as the enzymatic reactions controlled by them. But, one may think, it would be too naive to assume that nature will follow the simplicity of the illustrator Too naive? At least two cases were found where this simplicity is followed The cases were the known reactions leading to the synthesis of tryptophane or histidine, the two essential amino acids (review in [14]) When the sequences of the corresponding genes in the chromosomes were determined by transduction analysis, these sequences were found to be the same as the known sequences of enzymatic reactions controlled by them (review in) One cannot be sure why this is so but one feels that there is a logical beauty in this natural mechanism.

As mentioned throughout this book, all three systems are subject to disturbances. Obviously the systems are existent whether healthy or ailing a self reproducing gene molecule is a chemical reality whether it is a "normal (so called *wild*) or a mutant gene But it is easiest to notice the presence of a gene or a system which was taken for granted, when it changes or fails. Thus, the bulk of our knowledge about the three systems comes from the studies related to mutant genes which fail to perform their "normal" functions. The effects of such genic failures upon the third (peripheral) systems are known as *genetic blocks*

Consider for instance a mutation, in which the gene controlling the enzymatic reaction C → D (Figure 7) mutates to its inactive variety (*allele*) the corresponding enzyme will not be produced and the reaction between C and

D will be blocked The consequences of this *block* for the cell depend upon *many factors. The product D might be* of some small significance in natural selection (e.g., some unimportant pigment) in that case the mutant and the strain it originates may survive without this product for a long time. On the other hand, the product D may be essential for the life of the cell, as in the case of an amino acid or a vitamin. In such a situation it is said that the cell has acquired *an absolute requirement* for the metabolite D The cell can still live provided the metabolite D is supplied and can reach the inside of the cell. It may also happen that the reaction C→ D is blocked not completely but partially some of the product D is manufactured, but not enough of it. Such case is referred to as a *partial requirement* and such a mutant, in laboratory jargon, is called a *leaky mutant* But suppose that in a mutant which has an absolute requirement for substance D this substance cannot be supplied, or if supplied, cannot reach the interior of the cell. In this case, the cell will die: it is one example of *a lethal mutation*

The example of the sequence of enzymatic reactions as represented in Figure 7 is a very simple one. More often than not, the reactions are likely to pursue a veritable maze with by passes, side entrances and exits, and traps, as suggested by an imaginary example shown on Figure 8 to make things worse (for a scientist) some steps may be reversible and some not, some slow and some fast some end products may inhibit the activity of the preceding enzymes (so called *feed-back control*) so that not too much of the end product be made, etc. All these things complicate the study For example, a scientist will produce a block between *D* and *E* hoping that this will result in an absolute requirement for *E*, to his dismay the cell may outwit him by starting to manufacture *E* through a by pass B→F→G

→E. This by-pass mechanism might have been produced only at this particular moment, by the use of so-called adaptive enzymes, or it might have been there all the time, although normally not used One must not forget that the cell, though most economical, also believes in insurance and is prepared to meet all sort of emergencies.

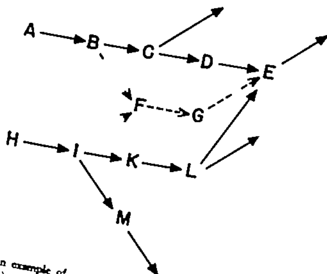


FIG. 8. An example of complex metabolic pathway (See text for explanation)

Consider another example A scientist notices that after a certain mutation the cell starts to manufacture product M which it did not manufacture before. One may think that a new enzyme to make M has been produced by the mutation. Actually the story may be entirely different Suppose that the reaction $I \to K$ occurred so much faster than $I \to M$ that normally all of I is requisitioned and there is no chance of it ever going to M But if a mutation produces a block between I and K then I will now be available

for conversion to M and a deceiving situation results.

Two other deceiving cases, where the inability to utilize citrate or to ferment lactose was due to the fault of permeability and not to the lack of enzymes, were mentioned previously

Despite these difficulties, the genetic blocks proved most useful for the unraveling of pathways of synthesis in the living systems (reviewed in [14]) The organisms most used were the mold Neurospora (Beadle, Tatum and others) and bacteria (Davis, Lederberg * Ryan and others) however there is little doubt that the information gained here is in most cases applicable also to higher organisms, including man. One example of the reasoning used in such a study will be explained below with reference to a simple scheme shown on Figure 7 (review in [12])

Let us assume that the sequence $A \rightarrow B \rightarrow C \rightarrow D$ is unknown, all one knows is that A is somehow converted into D One produces (e.g by irradiation) several different mutants which cannot manufacture D but can live if the latter is supplied. One of such mutants may accumulate a hitherto unkown substance C if given this substance, the mutant cannot utilize it to make D The suspicion is that G is an *intermediate* in the synthesis of D (a precursor of D) the substance C accumulates because it keeps on being manufactured by the cell but cannot be removed by conversion to D the block in this mutant is then, most likely between C and D Another mutant may accumulate not C, but a new substance B it cannot make D if B is given, but can make it if C is supplied. This points to B as an intermediate *before* C the block is now only between B and C.

By this painstaking research many synthetic pathways have been clarified and many hitherto unsuspected inter

* Nobel prize 1958

mediates discovered. It goes without saying that this knowledge is of utmost importance, not only for the basic understanding of the chemistry of life but also for many practical purposes the knowledge of the steps in natural synthesis leads to *logical* rather than empirical, designing of means to interfere with them (analogues carcinostatic agents) or to remedy them, if deficient. An aesthetic example of such a pathway is illustrated on Figure 9 (biosynthesis of threonine and methionine.)

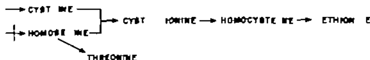


FIG. 9 Biosynthesis of threonine and methionine (from [144])

In the above discussion it was tacitly implied that the mutation of one gene can abolish only the action of *one* enzyme. Is this necessarily so? It is, according to the so-called *one-gene-one-enzyme hypothesis* (review in[11]) The proponents of the hypothesis quote numerous examples of *one* gene producing (or failing to produce) only *one* enzyme. The hypothesis was very attractive and therefore was promptly attacked by other scientists who were eager to point out examples to the contrary such as one mutation abolishing two of more enzymatic reactions at once. One mutation, for example, can abolish the synthesis of both threonine and methionine. "This—the proponents reply—is merely due to the complexity of the situation. Just take one look at Figure 9 again. obviously a mutation blocking *one* enzyme producing homoserine, will result in the failure to make *both* threonine and methionine. Thus far the proponents were able to answer all the cases of criticism, except the one that thus far the term gene" itself is not well defined from the biochemical point of view

In all of the above discussion, the mutations mentioned frequently were the *loss mutations*- some ability which the wild strain had, became lost The question frequently asked is: Are *all* mutations loss mutations? If they are, how can mutations be the source of raw material for evolution?

The biochemist s answer to the first question is the usual one we don t know A mutation may appear to be a *gain* mutation, such as a gain in an enzyme or in a "new" meta bolic pathway actually as we have discussed above, this new" enzyme or pathway might have been there all the time but was inhibited or inactive: it might become active by a *loss* of inhibitor a *block* in a normal pathway or in a side-exit. One must not forget that a new functional en zyme requires a new very specific and complex unit of genetic information, and an injury to a gene is more likely to destroy a unit of information than to create one from nothing The last word on this important subject has not yet been said however one must repeat, any proof of the newness or gain is going to be difficult. This field of science is at best fifty years old, but the history of any species— probably more than a hundred thousand can one guarantee that what we regard as appearing new has not been stored in the genetic memory of the species?

More can be said about the second question how can loss mutations be the source of raw material for evolution? The mutation may result in a loss, but the degree of harm may be so small that the strain may survive for quite a while. In the meantime the conditions may change so that the lost substance is no longer necessary this would make the mutant as good as the wild strain. Or even better In many cases one could demonstrate (in bacteria) that in the presence of the metabolite in the medium, the mutant which lost the power to manufacture this metabolite out *grows the wild strain which can manufacture it.* This

seemingly paradoxical situation may occur because the wild strain still keeps on manufacturing the metabolite, thus wasting time and energy on an unnecessary synthetic process; on the other hand, the "handicapped" mutant simply takes the ready metabolite from the medium. Some scientists believe that this is how parasitic species (requiring very complex media) came into existence; such species have lost the ability to manufacture many metabolites but survive very well because in the course of evolution the hosts which supply these metabolites became readily available. We ourselves have lost the ability to synthesize many metabolites, such as the so called essential amino acids and some vitamins, however we do not worry about it because we are parasites on other animals and on plants.

Many mutations are reversible; the process, called *reverse mutation* or *back mutation* may partially or completely restore the ability lost by the first (forward) mutation. If one investigates the metabolic pathways restored by the reverse mutation, it will be found that in many cases such a pathway is the original pathway of the wild strain, nothing seems to be gained or lost by the full cycle "forward reverse. But in certain cases the mutations is not to the original but to an entirely different pathway[183] in such a case the cycle may have an evolutionary advantage.

Well, anyhow possible or impossible, evolution occurs, and since nothing better than mutation was ever discovered as a raw material for evolution, we may as well accept it for the time being But another question is whether or not the evolution proceeds by the slow accumulation of small mutations (*micro-mutations*) While the majority of geneticists believes that such is the case, few voices are also heard favoring explanation involving macro-evolution, that is large genetical upheavals (*macro-mutations*) which would change whole blocks of essential features at

once[184, 85] Just how the organism could avoid a biochemical mess in such an upheaval is not clear. However, one must admit that the instabilities (high mutabilities) of whole blocks (if not all) of genes at once occasionally do occur[186]; as mentioned in Chapter IV, instabilities may escape elimination by natural selection[2] and may play some rôle in evolution.

Chapter VII

HEREDITARY DEFECTS IN MAN*

IT HAS been mentioned in the previous chapters that, thanks to the similarity of essential metabolic pathways in all organisms, many of the findings in microorganisms apply also to man. The question of just how many will be answered when enough biochemical research will have been conducted on human cells in tissue culture, especially when grown in suspension, not unlike bacterial cells.† But for a physician it is of more interest to consider the organism as a whole: can we understand any of the hereditary defects in man?

We can, but thus far only a few defects. To be sure, many defects are known to be hereditary, many of them are suspected to result from a biochemical disturbance (Table VII; review in [47, 48]). But in most cases we cannot put our finger on the exact chemical nature of the trouble. The studies in humans are understandably slow: there is the difficulty of obtaining old family records, hu

* In this book the term "hereditary" will be used rather than inborn or congenital. The latter two terms are broader, including not only hereditary defects but also defects in embryonic and foetal life.

† In general, the studies of human cells in tissue culture hold great promise for the future of human genetics. Pure strains from single cells can be grown and genetic differences between individual strains determined. Human cells *in vitro* can be subjected to radiation and the induced chromosomal abnormalities studied; undoubtedly other mutagenic agents will also be applied before long.

TABLE VII
HEREDITARY DEFECTS IN MAN

Name of Disease	*Biochemical Symptoms*	*Reference*
Albinism	Failure to manufacture melanin	109
Alkaptonuria	Failure to oxidize homogentisic acid	175 169
Cystinuria	Abnormal excretion of cystine, lysine, arginine and ornithine; urinary calculi	170, 171
Galactosemia	Failure to convert galactose-1-phosphate into glucose 1-phosphate	181 182
Gaucher's disease	Deposition of abnormal amounts of kerasin in the cells of reticuloendothelial system	109
Glycogenosis	Accumulation of glycogen in the liver heart and spleen	109
Gout	Elevated blood uric acid and deposition of sodium biurate in the tissues	109
Hemolytic anemia (spherocytosis)	*Defect in glycolysis*	*179*
Hemophilia	Missing blood clotting factor	109
Hypercholesterolemia	Elevated blood cholesterol	109
Hypophosphatasia	Abnormal mineralization of bone; diminished alkaline phosphatase activity increased urinary excretion of phosphatidylethanolamine	174
Infantile amaurotic idiocy	Accumulation of sphingomyelin in the brain cells	109
Pentosuria	Excretion of pentose sugars in the urine	109
Phenylketonuria	Failure to convert phenylalanine to tyrosine	177 178
Porphyria	Excretion of the porphyrins in the urine and feces	173
Sporadic cretinism with goiter	Defective synthesis of thyroid hormone	180
Tyroketonuria	Failure to convert p-hydroxyphenylpyruvic acid into 2,5-dihydroxyphenylpyruvic acid	109
Wilson's disease	Defective copper metabolism	172

man reproduction is slow and, above all, there is the difficulty of experimenting with living human tissue. In the few cases which we do understand, the disturbances turned out to be simple genetic blocks caused by mutations, just as the ones that occur in microorganisms described in the previous chapter These cases are discussed below

The best understood blocks in man concern the metabolism of the amino acid phenylalanine. In the normal course of events, the excess of phenylalanine from the diet is converted into tyrosine some of the latter is utilized as such and the excess is changed along two metabolic pathways (Figure 10) In one of these pathways, tyrosine is converted by the enzyme tyrosinase into 3,4-dihydroxy phenylalanine (called DOPA) and then by a series of reactions—into the pigment *melanin* Now some rare individuals, the *albinos* are unable to produce pigment—a striking deficiency early recognized as hereditary defect. Upon closer investigation it was found that the albinos lack the enzyme tyrosinase. One assumes that, as in the cases studied in microorganisms, one deals here with a simple genetic block (A on Figure 10) due to a mutation.

In the second of the metabolic pathways, the excess tyrosine is normally converted into p-hydroxyphenylpyruvic acid, then to 2 5-dihydroxyphenylpyruvic acid and then to homogentisic acid (alkapton) which is eventually metabolized to CO_2 and water (Figure 10) However in some rare individuals the homogentisic acid is not further metabolized and is excreted as such in the urine- the latter on standing turns black due to the oxidation of homogentisic acid In later life, the patients may also develop black pigmentation of the cartilage (ochronosis) probably also due to the oxidation of homogentisic acid. The disease was recognized as being hereditary (a simple recessive gene) by the brilliant work of Garrod who was also the first

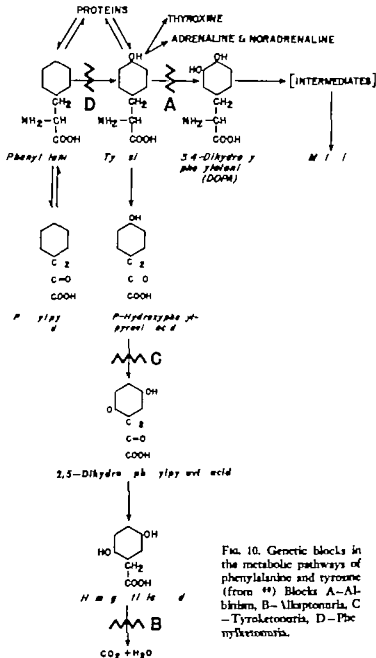


Fig. 10. Genetic blocks in the metabolic pathways of phenylalanine and tyrosine (from [44]) Blocks A–Albinism, B–Alkaptonuria, C–Tyroketonuria, D–Phenylketonuria.

to develop the concept of the *inborn errors of metabolism* The disease was called *alkaptonuria*, it was the first biochemical mutant recognized as such. The inability to metabolize homogentisic acid was eventually traced to the fact that the alcaptonurics actually lack the enzyme homogentisic acid oxidase which is present in the blood of normal persons again, we are dealing here with a simple genetic block (B on Figure 10) due to a mutation.

A disease called *tyrosinosis* is characterized by the excretion of large amount of p-hydroxyphenylpyruvic acid. The disease is so rare that one cannot even be sure that it is hereditary One suspects that the block prevents the conversion of p-hydroxyphenylpyruvic acid to 2 5-dihydroxy phenylpyruvic acid (C on Figure 10)

Returning to the discussion of phenylalanine, one notices that the excess of this amino acid can also be converted into phenylpyruvic acid. Normally this product does not appear in the urine it does, however in certain patients, who strangely enough, are also mentally deficient. The disease phenylketonuria, was found to be hereditary (a single recessive gene) The connection with the mental deficiency is still not clear but the biochemical side is easier to comprehend it was noticed that phenylalanine itself piles up in the blood of the patients also they are often albinos. These two facts indicate that the block is between phenylalanine and tyrosine (D on Figure 10) the increased level of phenylalanine results then in the appearance of phenyl pyruvic acid in the urine It is of great interest to note that the condition of mental deficiency could be and has been alleviated by limiting the amount of phenylalanine in the diet (review in)—a tangible dividend from chemical genetics.

Another important and well worked out metabolic pathway is that of glycolysis, the anaerobic breakdown of glu

cose. In a group of hereditary diseases called *congenital hemolytic anemias* glycolysis in red blood cells has been found defective (review in[11]). A detailed study points again to a simple genetic block: the enzyme involved seems to be either enolase which converts 2-phosphoglycerate to phosphoenolpyruvate, or the phosphofructokinase which converts fructose-6-phosphate into fructose-1,6-diphosphate.

Sporadic cretinism with goiter appears to be a hereditary disease caused by the arrest at any stage of the synthesis of the thyroid hormone (review in[1]). Three mechanisms have been studied. In one, the block due to a lack of the oxidative enzyme causes a failure in such individuals to produce mono- and diiodotyrosine from iodide and tyrosyl residues. In another mechanism, the diiodotyrosine molecules fail to couple with sufficient speed into thyroxine, presumably due to the failure of the coupling enzyme. The third mechanism deals with the catabolism of the above mentioned precursors, mono- and diiodotyrosine. During the process of thyroglobulin degradation the above precursors escape from the thyroid gland into the blood, and normally are rapidly deiodinated by the enzyme iodotyrosine deshalogenase whereby the iodine is made available for reutilization. If the enzyme fails (due to a genetic block) the iodinated precursors escape to the urine and a serious iodine deficiency may result.

Galactosemia is a hereditary disease characterised by a failure in the metabolism of galactose (review in). In the normal metabolic pathway, galactose is first converted by means of adenosine triphosphate (ATP) into galactose-1-phosphate; the latter, by means of uridine diphosphate glucose (UDPG), is converted into glucose-1-phosphate which enters as such into the normal pathway of utilization of glucose. The enzyme involved in this last

reaction, P-galactose-uridyl transferase, was found to be lacking in the erythrocytes and the livers of galactosemic patients[3] This genetic block results in an accumulation of galactose 1 phosphate which seems to produce tissue damage and growth inhibition. The exclusion of galactose should prevent accumulation of galactose 1 phosphate it was found that indeed the galactose free diet produces a reversal and, often, complete clearance of all symptoms, with restoration of normal health.

In all the above considerations we referred to the "failure of the enzyme" or "lack of the enzyme." However the lack of the enzyme does not necessarily mean that the molecule of the enzyme (or of the protein in general) is missing altogether: it may often mean that it is defective i.e. changed so as to be partially or totally inactive. Just how much has to be changed to cause inactivation? What is the smallest change in the protein which the mutation has to produce to make itself drastically felt?

One example of the answer to these questions has been provided by a study of a hereditary disease called *sickle cell anemia*. The disease, caused by a single mutant gene, is characterized by the presence of defective erythrocytes which are in the form of sickles. The defect was traced to defective hemoglobin and the problem was to determine what is the chemical nature of the difference between this defective and the normal hemoglobin.

Pauling and his colleagues subjected the two kinds of hemoglobin to electrophoresis and found that the two have different electric charges.[11] This behavior suggested some difference in the amino acid composition. But how many were different? And which ones? A molecule of hemoglobin has some 600 amino acids, of 19 different kinds, and the problem might have appeared hopeless. It was solved eight years later by Ingram[3] who broke the mole

cule in half and then into 29 smaller fragments (peptides) When such fragments from normal hemoglobin were compared with the corresponding fragments from the defective hemoglobin, all were identical, *except one* And in this fragment, all amino acids were identical *except one* Thus, the disease was caused by a change of one amino acid in 300 (glutamic acid replaced by valine) This is all the mutation had to do to make itself seriously felt. One is tempted to speculate that the corresponding change in the hereditary determinant (DNA molecule) was just as small perhaps it was a change in a single nucleotide.

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All this is a mere beginning But the beginning *has* been made. And one is inclined to agree with Albert Einstein " that the most incomprehensible thing about nature is that it is comprehensible

APOLOGY

The writer feels that he owes several apologies.

First, an apology to the reader For the book being so long yet failing to offer real answers to many formulated queries. The writer's excuse is that the subject is but an infant, if we have become able to ask nature embarassing questions, it is already a crack in the wall. And an apology for the book being so short. The discussion of many hereditary diseases and of the cancer field has been omitted, partially because these subjects belong to special treatises, and partially because their connection with the chemistry of heredity is merely the object of *future* study

The writer also owes an apology to the scientists whose painstaking research he had no chance to describe in a book of this size. The reader can be counted upon to realize that in the background of every spectacular achievement there are hundreds of publications which prepare the soil.

But there is still another apology a more grave one an apology for the attempts, by all chemical geneticists, to tamper with nature's most closely guarded secrets. A control, at will, of the heredity of virulent microorganisms and of man may result from these attempts. Like the release of atomic energy the control of heredity may be utilized for the total destruction or for the benefit of our species.

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